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EFFECTS OF PROTEIN DIETS ON GROWTH AND HAEMATOLOGY OF *SPERATA SEENGHALA* FROM HEAD PUNJNAD PAKISTAN

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Abstract

Present research work was conducted to evaluate the effects of various crude protein (CP) diets on the growth and haematology of catfish, *Sperata seenghala.* Experimental trial was conducted in Aquaria each having water capacity of 70 L (In triplicate) for 90-days. Fish was fed at 5% of wet body weight, twice a day. Five feeds were formulated comprising of 30%, 35%, 40%, 45% and 50% crude proteins, were designated as T_1, T_2, T_3, T_4 and T_5. However, T_6 was designated as Control group containing fish meal (100%). *Sperata seenghala* fed with T_4 (45% CP) has shown higher average final body weight (47.12±3.38) representing significantly (P<0.01) higher growth than T_1 (30% CP), T_2 (35% CP) T_3 (40% CP) and T_5 (50% CP). While lowest average final body weight was observed with fish feed T_6 (F.M). Live weight gain (LWG) was observed maximum (31.74±0.98) in feeding group T_4 (45% CP) while least live weight gain (LWG) 10.33±1.88 was detected in feeding group T_6 (F.M). Similarly, percent live weight Gain (%LWG) was determined maximum 206.37 in T_4 (45%CP) representing better growth in T_4 (45%CP). Fish efficiently assimilated feed of T_4 (45% CP) and displayed least feed conversion ratio (FCR) as 4.10 (p<0.01) while maximum values of FCR were determined in T_6 (9.19) and T_1 (6.60). Numerous haematological parameters such as WBCs, LYM, MON, GRA, RBCs, HGB, RDW, MCV, MCH, MCHC, PLT and MPV were studied which showed significant effect (p<0.01). Fish with T_4 (45%) CP represented maximum haemoglobin Hbg (8.25±0.21) showing highly significant values (p<0.01). Maximum RBCs were observed 2.39±0.09 (10^6/µL) in T_6 while minimum RBC value was observed as 1.69±0.13 (10^6/µL) in T_4. Minimum WBCs (106.64±1.38) were observed with CP T_4 (45%) showed highly significant relation (p<0.05), representing unstressed conditions and good health status of fish. The order of WBC's values observed was $T_1 > T_6 > T_5 > T_2 > T_3 > T_4$.

Key words: *Sperata seenghala*, Crude protein, Growth, Feed conversion ratio, live weight gain, red blood cells, haemoglobin, white blood cells, haematology.

INTRODUCTION

The Bagridae, a family of catfish that is native to Africa (*Bagrus*) and Asia (All other Genera) from Japan to Borneo (Nelson, 2006). Large Bagrids are significantly used as food fish. Some species are kept as aquarium fishes (Nelson, 2006). Resistance to diseases, high fecundity and easy production in confinement has made it significant commercially (Noor El-Deen *et al.*, 2014; Ljubobratovic *et al.*, 2015). *Sperata seenghala* is also known as Giant River Catfish. It is a commercially very significant species and also has a significant contribution towards the total domestic fish production in South Asia including Pakistan. It is highly preferred by customers because of having good quality, delicious flesh and low quantity of intramuscular skeleton (Agbayani, 2004). It is a famous fish species to capture because it draws much higher price than carps (Tripathi, 1996). From aquaculture standpoint Giant River Catfish has wide range of tolerance regarding salinity and oxygen reduction (Pethiyagoda, 2005). It is good fighter fish and delivers good sport fishing. It spawns two times a year in its natural habitat from May to July and from September to November (Talwar and Jhingran, 1991; Rahman *et al.*, 2005b, 2011).

Although regional differences in food components has made feed formulations quite difficult but in aquaculture, nutrition is such a diversified and vital ground where a lot of work has been done and in future there is an immense scope of research in it (Eroldogan *et al.*, 2004; Gomez-Requeni *et al.*, 2004; Eroldogan *et al.*, 2006a). Diet containing extensive proteinaceious material is used for energy that outcomes to enhance nitrogenous waste like ammonia removal (Cho and Bureau, 2001; Kim *et al.*, 2004). However, when there is an insufficient protein in diet, it results in reduction of growth (Kim *et al.*, 2004).

It is a fact that fish feed accounts for at least 50–60% of the total price of fish production (Gabreil *et al*., 2007). If fish meal price is increased in markets then production cost of farmed fish will also be increased (Hardy, 2010). Numerous types of feed have been explored to find alternates of fish meal in fish feed (Muin *et al.*, 2014; Muin *et al*., 2015). For fish feed, fish meal is considered as best protein source, because of having high quantity of quality protein, high quantity of phospholipids, vital fatty acids, essential amino acids and higher level of its digestibility and palatability (Barlow, 2003). Most studies reported that the protein prerequisite of catfish extended from 35% to 45% (Jamabo and Alfred-Ockiya, 2008). Furthermore, dietary protein is basic ingredient in fish diet that not only support growth but also can correspondingly enhance the feed expenditures (Ahmad *et al.*, 2012).

Blood is a significant connective tissue which is involved in the regulation of several metabolic accomplishments of an organism. As for as physiology and pathology of fish is concerned, normal ranges for various blood parameters in fish have been recognized (Xiaoyun *et al.*, 2009). Examination of blood indices has proved to be a valued procedure for determining the health status of fish which delivers trustworthy information on metabolic disorders, shortages and long-lasting stress status (Bahmani *et al.*, 2001). Hematological parameters of fish also represent the reaction of fish to ecological and biological elements (Fernandes and Mazon, 2003).

Assessment of haemogram comprises the determination of total erythrocyte calculation (RBC), total white blood cell amount (WBC), Haematocrit (HCT/PCV), haemoglobin concentration (Hb), erythrocyte indices mean corpuscular volume (MCV), Mean corpuscular Haemoglobin (MCH), Mean corpuscular Haemoglobin concentration (MCHC), White blood cell differential calculation and the assessment of stained peripheral blood films (Campbell, 2004).

Study of Erythrocyte parameters can be used for diagnosis of anemia or stress polycythemia, while Leucocytes calculations may disclose leucopenia or leukocytosis, advising possible immune function modifications (Huffman *et al*., 1997). White blood cells, the principal line of immunological defense, provide significant illustration of defense cells throughout the body (Tavares-Dias and Moraes, 2004; Tavares-Dias and Moraes, 2007; Affonso *et al*., 2007; Pavlidis *et al*., 2007). One of the basic ways to evaluate the immune system is to determine variations in the number or appearance of circulating white blood cells (WBC) in fishes, also the numbers of Leucocytes and Thrombocytes are vital indicators of health status of fish and in numerous cases, these are also supportive for evaluating the Immune system (Tavares-Dias and Moraes, 2004). Leucocytes (WBC) behavior is adaptive in nature and may fluctuate according to their environment (King *et al.*, 2018). The standard value of fish leukocyte (WBC) is 20,000-150,000 cell/mm^3 (Moyle and Cech, 2004). Certain haematological parameters such as hematocrit (HCT), erythrocyte counts (RBC), and hemoglobin (Hb) concentrations are most enthusiastically determined in field and hatcheries conditions (Bhaskar and Rao, 1990).

A mass of intrinsic and extrinsic aspects causes variations in haematological data such as water quality, temperature (Magill and Sayer, 2004), nutritional state (Svetina *et al*., 2002; Lim and Klesius, 2003), stress (Cnaani *et al*., 2004), culture conditions (Hrubec *et al*., 1996), the cycle of sexual maturity, photoperiod (Leonardi and Klempau, 2003), age or sex of the fish can affect some blood value. Furthermore, blood parameters of fish are exceedingly sensitive to ecological variations (Sheikh and Ahmed, 2016).

The number of white blood cells (WBC) and thrombocyte count (TC) may vary relating several environmental stimuli such as infection, as well as the age of fish to species characteristics or nutritious alterations (Romano *et al*., 2017). Active species have higher values of haematological parameters in contrast to less active forms (Svobodova *et al*., 2008). Generally higher number of red blood cells (RBC) is linked with fast movement and high activity in wild and farmed species having streamlined bodies (Fazio *et al.*,2013, 2016).

Hematocrit is used to determine comparison between red blood cells (erythrocytes) and plasma to demonstrate the total percentage of erythrocytes to the total volume of blood in body. Hematocrit values are affected by number and size of red blood cells. Higher hematocrit counts may reveal an absolute increase in count of erythrocytes or reduction in plasma volume ultimately (Wennecke, 2004). Seasonal variations in haemoglobin (Hb) are actually associated with fluctuations in temperature of water and variations in oxygen content of water (Akinrotimi *et al*., 2011).

Keeping in understanding the forthcoming scope of this species, more comprehensive attempt was essential to familiarize Catfish *Sperata seenghala* in Pakistan. Present research work was designed on feed formulations containing different crude proteins (CP) to promote its culture in more advantageous and conveniently more comprehensive way. Major objective of this study was to evaluate effects of various proteinaceious diets (CP) on growth and haematology of *Sperata seenghala* from local feed.

MATERIALS AND METHODS

Five feeds with different crude protein (CP) levels were formulated comprising of 30%, 35%, 40%, 45% and 50% crude proteins(CP) and were designated as T_1, T_2, T_3, T_4 and T_5. However, T_6 was designated as Control group containing fish meal (100%) (Table 1). Feed ingredients were meticulously mixed by means of a mixing machine and were lastly ground to powdered form for more easy consumption. All feed mass was shaped in accordance with contemporary good manufacturing procedures at Bahauddin Zakariya University's feed mill unit located in Animal House of the University. Feed Ingredients were ground with a locally intended hammer mill (Tufail industries, Faisalabad, Pakistan) furnished with 1.5 mm screens.

This experimentation was conducted for 90 days in Aquaria (In Triplicate) at Fish Research Laboratory of Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan. Fish samples were collected from Head Punjnad (Punjab, Pakistan) and were conveyed in oxygen saturated polythene bags to Fish Research Laboratory of Institute of Zoology located at Bahauddin Zakariya University, Multan, Pakistan.

After seven days of acclimatization under laboratory situations, normal and healthy fish samples were weighed. Fish samples were stocked randomly at the rate of 15 fish per aquarium with their average initial weight extending from 15.21±1.49 to 15.87±1.16g and average initial length extending from 11.20±1.19 to 11.58±0.95cm. Eighteen aquaria were settled in three groups, with each aquarium having water capacity of 70L in model of triplicate. Aquaria were filled with tap water and the water in the aquaria was replaced after every third day with fresh water at 1:4 (Old: Fresh) ratio till the completion of the experiment. All treatments were fed with 5% wet body weight during whole experiment. Fish samples were fed twice a day from 08:00-09:00 a.m. and 06:00-07:00 p.m.

Fish were weighed and measured fortnightly to assess the weight and length increments throughout experiment. An electronic Digital balance was used to measure the wet body weight (g) while a measuring ruler adjusted on wooden bar was used to measure the total length (cm) and other morphometric parameters.

The fish mortality rate was also determined on fortnightly basis. Growth Performance Parameters like Live Weight Gain (LWG), Percent Live Weight Gain (% LWG), Total Length Gain (TLG), Percent Total Length Gain (% TLG), Condition Factor (K), Feed Conversion Ratio (FCR), Protein efficiency ratio (PER), Percent survival rate (% Survival), Daily growth rate (DGR), Specific growth Rate (SGR) and the Production were calculated by using the standard formulae.

The physical indices (Water quality parameters) like temperature, dissolve oxygen (DO) and pH were measured fortnightly on depth of 10 cm by using oxythermometer (WTW Oxi 197i, WTW, Weilheim, Germany, precision: 0.01 1C and 0.01mg/L), DO-meter and pH meter (WTWpH 330i, precision: 0.01) respectively. Nitrite, nitrate, ammonia and phosphate levels were measured with help of Spectrophotometric methods according to APHA (1992) (Table 3).

At the end of the trial, five fingerlings were randomly sampled from each aquarium having different CP as 30% (T_1), 35% (T_2), 40% (T_3), and 45% (T_4), 50% (T_5) crude proteins (CP) and T_6 (Fish Meal). Blood samples were drawn by direct puncturing the heart by using 3 ml heparinized plastic syringes. 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).

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 with help of haematological analyzer (Medonic).

Collected data based on randomized design was subjected to (one-way analysis of variance) at 0.01 significant level followed by Duncan's Multiple Range Test (DMRT) for segregation of the means. Results were also quantified as Mean ± SD by using Microsoft Excel sheet on Windows 2010. Differences in haematological and absolute growth indices were evaluated by using SPSS statistical software version 16.0 to differentiate differences in mean values.

Results

Data showing effect of various crude proteins (CP) level on growth performance of *Sperata seenghala* are given in table (2). Present study has shown that *Sperata seenghala* fed with T_4 (45% CP) has shown higher average final body weight (47.12±4.30) representing higher growth than T_1 (30% CP), T_2 (35% CP) T_3 (40% CP) and T_5 (50% CP). While lowest average final body weight was observed with fish feed T_6 (F.M). Feed T_4 (45% CP) has shown significantly ($p<0.01$) better growth relating average final body weight in trial of 90 days in aquaria than T_3 (40% CP), T2 (35% CP) and T_1 (30% CP). During this trial average final body weight gain was found to be increased with increased crude proteins (CP) up to 45% (CP). Live weight gain (LWG) was observed maximum (31.74±0.98) in feeding group T_4 (45%) while least live weight gain (LWG) 10.33±1.88 was detected in feeding group T_6 (F.M) **(**Fig.1**).** Similarly, percent live weight Gain (% LWG) was determined maximum 206.37 in T_4 (45% CP) representing better growth in T_4 (45% CP) (Table 2).

Fish efficiently assimilated feed of T_4 (45% CP) and displayed least feed conversion ratio (FCR) as 4.10 while maximum values of FCR was determined in T_6 (F.M) and T_1 (30% CP). Protein efficiency ratio (PER) in increasing order $T_4>T_2>T_3>T_1$ demonstrated non-significant differences (P>0.01) while T_5 and T_6 showed significant differences with T_4. Specific growth rate (SGR) was found maximum in T_4 (0.540) while least values of SGR Specific Growth Rate were observed in T_6 (0.246). A significant difference was observed in SGR values in all the feeding groups (T_1, T_2, T_3, T_4, T_5 and T_6). Fulton Condition factor (K) values were observed maximum in T4 (0.848±0.17) while other feeding groups represented Condition factor values below T_4. Maximum production was perceived in T_4 (CP 45%) while least biomass production was determined in T_6 (F.M.) (Table 2).

Daily growth rate (DGR) was observed maximum 2.293 in T_4 (45% CP). Order of Daily growth rate (DGR) was observed as 2.293(T_4,45% CP)>1.579(T_3,40%CP)>1.332(T_2,35% CP)>1.046(T_1, 30%CP)>1.092 (T_5, 50%CP)>0.739 (T_6, F.M). Maximum survival percentage was observed 100 Percent in T_3 (CP 40%) and T_4 (CP 45%) while maximum mortality rate was detected as 93.33 % in T_5 (CP 50%) and T_6 (F.M) (Table 4). All the water quality parameters were observed optimum for fish growth (Table 3). Fortnight increase in wet weight and total length was expressed in figure 1 and 2.

The RBC's count as haemoglobin concentration (g/dl), MCV-mean Corpuscular Volume (μm3), MCH-mean Corpuscular Haemoglobin, MCHC-mean Corpuscular Haemoglobin Concentration (g/dl) and HCT-hematocrit (%), RDW-red blood cell distribution width (%) and RDW-SD -red blood cell distribution width in *Sperata seenghala* were measured relating various crude proteins T_1, T_2, T_3, T_4, T_5 and T_6 shown in Table 4.

RBCs values were observed as 2.39±0.09 (T_6), 2.358±0.13 (T_5), 2.128±0.13 (T_1), 1.96±0.08 (T_2), 1.75±0.06 (T_3) and1.69±0.13 (10^6/μL) (T_4). Maximum RBCs were observed 2.39±0.09 (10^6/μL) in T_6 while minimum RBC value was observed as 1.69±0.13 (10^6/μL) in T_4.

The haemoglobin (Hgb) value was observed as 8.25±0.21 (g/dl) (T_4),7.5±0.35 (T_3), 6.92±0.20 (T_2), 6.698±0.35 (T_1), 6.62±0.20 (T_6) and 6.618±0.41(T_5) representing maximum haemoglobin (Hgb) value in T_4 (45% CP) and minimum haemoglobin (Hgb) value in T_5 (50% CP).

The MCV values were observed as 195.06±17.31(T_3), 177.78±5.46(T_4), 176.8±5.49 (T_2),174.48±4.68(T_5), 174.12±3.41(T_6) and 171.22±4.55(T_1) (μm^3). Maximum value was observed with 40% CP while least value was measured with 30% CP.

The MCH values were determined as 39.6±2.39(T_4), 39.36±1.99(T_3), 38.02±1.17(T_1), 37.62±0.88(T_6), 36.7±0.65(T_5) and 36.04±1.21(T_2). Maximum value of MCH was determined with 45% CP and minimum was observed with 35% CP. The MCHC values were measured as 23.54±1.23 (T_4), 22.266±0.79 (T_1), 22.1±0.38 (T_6), 21.28±0.23 (T_5), 20.3±1.50 (T_3) and 19.24±0.94 g/dl (T_2). T_4 (45% CP) showed maximum value while T_2 (35% CP) represented the minimum value.

RDW % values were observed as 11.02±0.46 (T_3), 10.52±0.25 (T_2), 10.32±0.28 (T_4), 9.9±0.31 (T_5), 9.78±0.26 (T_6) and 9.62±0.21 % (T_1) represented its maximum value with 40% CP and minimum value with 30% CP. RDW-SD values were observed as 175.98±8.13 (T_3), 88.08±4.17 (T_4), 83.8±3.48 (T_2), 80.14±1.22 (T_6), 80±0.99 (T_5) and 79.632±1.99μm^3 (T_1), represented maximum value with 40% CP and minimum value with 30% CP.

The WBCs count in *Sperata seenghala* under the influence of various crude proteins T_1, T_2, T_3, T_4, T_5 and T_6 shown in Table 1. The WBCs values were observed as 169.72±0.88 (T_1), 140.36±0.73 (T_6), 136.62±1.30 (T_5), 127.48±1.57 (T_2), 124±0.63 (T_3) and 106.64±1.38 (T_4) (10^3/μL). Maximum value of WBCS was observed 169.72±0.88 (T_1) with 30% CP while minimum was observed 106.64±1.38 (T_4) (10^3/μL) with 45% CP. The order of WBC's values observed was $T_1> T_6> T_5> T_2> T_3>T_4$.

The lymphocytes count observed was 149.43±1.90 (T_1), 119.63±1.23 (T_6), 118.76±1.22 (T_5), 117.15±1.68 (T_2), 113.02±1.27 (T_3) and 100.02±1.39 (T_4) ($10^3/\mu L$). Maximum count of lymphocytes was observed in T_1 with 30% CP and minimum was observed in T_4 with 45% CP. The order of observation of lymphocytes in different treatment groups was $T_1 > T_6 > T_5 > T_2 > T_3 > T_4$.

The monocytes count in 11.793±0.78 (T_6) 10.62±1.01 (T_1), 10.27±0.73 (T_5), 6.246±0.37 (T_2), 6.001±0.48 (T_3) and 3.83±0.22 (T_4) ($10^3/\mu L$). Maximum monocyte count was observed with T_6 (FM) while minimum was determined in T_4 with 45% CP. The monocyte count observation order was $T_6 > T_1 > T_5 > T_2 > T_3 > T_4$.

The granulocytes count was observed as 9.66±3.95 (T_1), 8.928±0.82 (T_6), 7.575±1.62 (T_5), 4.978±0.57 (T_3), 4.077±0.52 (T_2) and 2.77±0.17 (T_4) ($10^3/\mu L$) with percentage to total leucocytes as 6.36±0.53 (T_6), 5.68±1.79 (T_1), 5.54±0.88 (T_5), 4.02±0.65 (T_3), 3.2±0.49(T_2) and 2.6±0.16 (T_4). The granulocytes observation order was $T_1 > T_6 > T_5 > T_3 > T_2 > T_4$.
The thrombocytes (Platelets) count in *Sperata seenghala* under the influence of various crude proteins (CP) T_1, T_2, T_3, T_4, T_5 and T_6 shown in Table 4. Platelets count was observed as192.84±2.10 (T_6), 191.3±3.48 (T_5), 186.52±5.26 (T_1), 176.5±5.49 (T_4), 166.3±5.24 (T_3) and 120.28±1.74 (T_2) ($10^3/\mu L$). Maximum count of platelets was observed in T_6 (FM) while minimum was determined with 35% CP (T_2).
MPV (Mean Platelets Volume) values were determined as 6.56±0.196 (T_2), 6.34±0.19 (T_1), 6.26±0.94 (T_5), 6.26±0.14 (T_6), 6.08±0.23 (T_4), and 5.94±0.20 (T_3) (μm^3).
PCT-plateletcrit percentage values were measured as 0.169±0.014 (T_1), 0.119±0.005 (T_6), 0.112±0.008 (T_5), 0.107±0.003 (T_4), 0.09±0.004 (T_3) and 0.076±0.02% (T_2). Maximum value (PCT %) was observed with (T_1) 30% CP.
PDW percentage (platelets distribution width) was observed as10.92±0.23 (T_2), 10.28±0.46 (T_4), 9.174±0.34 (T_1), 9.082±0.183 (T_6), 8.96±0.169 (T_5) and 8.94±0.11 % (T_3).

DISCUSSION
Sayeed *et al.* (2008) reported that final body weight and specific growth rate of *Pangasius hypothalamus* increased with increased crude protein (CP) level in feed, protein range diverse from 26 to 40% CP diet which predicts that relatively higher crude protein (CP) levels are essential for improved growth and productivity, however higher growth in present study of *Sperata seenghala* was observed with 45% crude protein diet (T_4). Abdel-Tawwab *et al.* (2010) found parallel results to present study by observing highest growth with 45% crude protein diets. Malik and Naeem (2020) reported that FCR (feed conversion ratio) improved with increase in dietary protein moving from T_1 (20%) to T_3 (30%) concluding best growth with T_3 (30%) having contradictory findings with present study where maximum growth was observed with T_4 (45% CP)**.** According to Ali *et al.* (2014), higher growth was observed in *Sperata seenghala* fed with 40% crude proteins (CP) while in present study higher growth was observed in *Sperata seenghala* with 45% crude proteins (T_4). Present study also has contradictory findings with khan *et al*. (2018) that described Fish fed with 40% crude protein (CP) showed significantly ($P<0.05$) higher growth (1378.57±53.20g) as compared to 44% (CP) crude protein.

Biswas *et al.* (2019) has reported that the growth and average weight gain of Indian Butter Catfish *(Ompokbi maculatus*) increased significantly with increasing protein levels (CP) up to 35% representing positive correlation and decreased afterwards. A similar trend was also observed in numerous other fish species like *Takifugu rubripes* (Kim and Lee, 2009), *Ctenopharyngodon idella* (Jin *et al*., 2015), *Epinephelus akaara* (Wang *et al*., 2016), *Takifugu rubripes* (Kim and Lee, 2009), *Pangasianodon hypophthalmus* (Jayant *et al*., 2017) and *Oplegnathus fasciatus* (Kim *et al*., 2017), however these findings have contradictions with our present study on *Sperata seenghala* where growth has been increased with T_3 (40% CP) and T_4 (45% CP). The lower fish growth with low crude protein feeds can be due to the fact that pressure on body protein may possibly be high because of scarcity of proteins to conserve good growth and metabolism (Lee *et al*., 2001).

Present study has revealed that reduced growth has also been reported at high crude protein (CP) diets as in T_5 (50% CP) than T_4 (45%CP) specify that additional amount of crude protein leads to deamination and catabolism to deliver energy for maintenance (Jayant *et al*., 2017; Ye *et al*., 2016). Many other researchers have also made similar conclusions (Guo *et al*., 2012; Kim and Lee, 2009) parallel to present study.

Furthermore, it is a well acknowledged fact that fish does not have the capability to exploit additional dietary protein for protein production, with a large fraction of the dietary protein is exploited as energy source in metabolism (Wu *et al*., 2014). In present study, crude protein requirement observed in T_4 (45% CP) is rationally far from those of Jindal (2011) for Asian catfish (36% CP), Giri *et al* (2011) for yellow catfish (35% CP) and Jayant *et al* (2017) for Striped catfish (37.1% CP), but is closer (not same) to findings of Siddiqui and Khan (2008) for Stinging catfish (40–43% CP). Average final body weight and specific growth rate of the juvenile of slender walking catfish (*Clarias nieuhofii*) increased as dietary protein level was increased from 32% to 40% by Suphada and Anut (2008) but no progress in growth performance was detected at 44% crude dietary protein (CP) level which is not parallel to present study which showed higher growth with T_4(45%) crude protein in *Sperata seenghala*. The excessive protein content in the diet could reduce the growth performance of fish due to higher energy requirement for catabolism rather than for protein deposition. The decrease in weight gain, when the fish were fed excess level of dietary protein may also be because of a reduction in available energy for growth and due to inadequate non-protein energy necessary to deaminate the high protein feed (kim *et al*., 2002). The reduced growth rate and decreased protein utilization beyond requirement of dietary protein level is well documented in the past by several workers (Cho *et al.*, 2005; kim and Lee, 2005; Sa, *et al.*, 2006).

Ishtiaq and Naeem (2019) reported sufficient production and maximum growth in *Catla catla* fed by 25% crude protein diet in a polyculture system as compared to higher protein level like 30% CP which has contradictory findings with present study in which maximum growth was observed at higher protein level (45%).

Hematological parameters were also significantly affected by crude protein level. According to Khan *et a*l. (2018) hematological values erythrocytes (RBCs) leucocytes (WBCs), haemoglobin (Hb) level, monocytes and neutrophil were found to be highest with 40% (CP) crude protein and the lowest count of all above mentioned hematological parameters at 35% (CP) crude protein level. Their maximum activity was found in 40% CP crude protein level feed than 35% and lowest for 44% crude protein CP level that was contrary to present study of Sperata *seenghala* where haemoglobin (Hb) value was found maximum in T_4 (45% CP) and lowest WBCS was observed in T_4(45% CP) representing better growth and less stress condition. Maximum hematological activity was reported with 40% CP diet than 35% and lowermost for 44% CP diet (khan *et al*., 2018) having contradictory findings with *S. seenghala*. The fish diet containing 40% and 45% crude protein had significantly highest haemoglobin (Hb) concentration followed by 50% crude protein diet ($P<0.01$). Values of Hb content were reported intermediate at 35% crude protein diet, while poorest Hb content was assessed at lowermost level of crude protein diet (25%), however in present study of *S. seenghala* lowest hemoglobin value was observed in T_5 (50% CP) and maximum hemoglobin value was observed with T_4 (45%).

Haematocrit (HCT) values increased significantly with the increase in dietary crude protein levels from 25% to 45% protein (Khan and Maqbool, 2017). However, higher HCT value (38.26%) was reported for fish fed 45% protein diet, although the lowest HCT value (22.19%) was noted at the lowest protein level (25%) (Khan and Maqbool, 2017), while lowest HCT value was observed in present study of *S. seenghala* was observed with T_5 (50%).

Red blood cell counts (RBC) in fish fed various dietary protein levels also produced significant differences. Significantly highest RBC value (1.9×10^6 mm^3) was noted at 45% protein diet found parallel to present study of *S. seenghala*, followed by those receiving diet at 40% protein diet (P<0.05). Intermediate RBC values were recorded in fish fed other dietary protein levels, except those fed 25% and 30% protein diets, while significantly lowest RBC count values were obtained (P<0.05) at 25% and 35% crude protein diets. RBC and Hct values were reported to be significantly lower in Italian farmed rainbow trout than Turkish, as in present study of *S. seenghala* (T_4), conversely MCV, MCH, and MCHC were shown to have significant higher values.

WBC and Hgb have shown no significant difference (Faizo *et al*., 2016). Reduction in RBCs represents the anemic condition due to stress situations (Li *et al*., 2011). Amplitude of toxicants in diet causes hemolysis which results in dwindling in RBCs count (Kavitha *et al*., 2010; Saravanan *et al*., 2011). Hgb concentration in T_4 and T_3 treatment groups of fish was significantly higher than T_5 and T_6 groups, which represent good health status of fish while lower concentration represents that oxygen carrying capacity, may be influenced by treatment in water and pollution (Ahmed *et al.*, 2011; Nasir *et al.*, 2023).

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). In present study, RBCs count differs significantly on varying protein diets. A highly significant increase in RBCs and WBCs was observed in diet containing high proportion of crude proteins with T_6 (Fish meal), T_5 (50% CP) which is contrary to the conclusion of Yue and Zhou (2008) and Iqbal *et al.* (2016) as they observed low proportion of RBCs and WBCs in juvenile hybrid tilapia and juvenile *Labeo rohita* respectively but agreed with the findings of Nasir and Al-Sraji (2013) as they observed high proportion of RBCs fed at higher protein content diet instead of low protein content diet. Thus, higher the proportion of RBCs in blood, the fish is in stressful condition. However, RBCS and WBCS values were observed comparatively less with 45% CP (T_4) representing much better growth. Wepener *et al.* (1992) reported RBCs swelling may indicated by MCHC, which is ratio of blood Hgb as apposite to PCV, not influenced by blood volume or cells. In present study a significant lymphocytes count was observed with all CP diets. The order of observation of lymphocytes in different treatment groups was $T_1 > T_6 > T_5 > T_2 > T_3 > T_4$. Platelets indicate the disease process and disease resistance, present study observed the higher concentration in T_6 (FM), however significant platelets count was observed in T_1 (30%), T_3 (40%), T_4 (45%), T_5 (50%) while minimum count was observed in T_2 (35% CP).

Deficiency of good quality fish feed has harmful effects on growth rates, disease outburst, whole yield of fish and haematology (Fakunle *et al.*, 2013). Intensities of dietary protein and energy not only affect the growth, proximate composition but also affect digestive enzymes actions and plasma metabolites with haematology in numerous fishes (Yamamoto *et al.*, 2006).

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Table 1. Ingredient composition and proximate analysis of experimental feeds.

Ingredients composition	T_1 (30%)	T_2 (35%)	T_3 (40%)	T_4 (45%)	T_5 (50%)	T_6 (Fish Meal)
Fish meal	30	42	50	55	60	100
Corn glutan(60%)	01	02	03	04	06	---
Corn glutan (30%)	01	01	02	05	03	---
Rice polish	45	30	15	15	05	---
Sunflower	08	10	12	05	05	---
Wheat bran	08	08	10	05	05	---
Canola meal	01	01	01	02	05	---
Mustard cake	01	01	02	02	02	---
Soybean meal	01	01	01	03	05	---
DCP	01	01	01	01	01	---
Minerals	01	01	01	01	01	---
Oil	02	02	02	02	02	---
Total	**100**	**100**	**100**	**100**	**100**	**100**

Table 2. Growth performance, survival, production and feed utilization of *Sperata seenghala* reared at six different feeds for 90 days in aquaria.

Parameters	T_1 (30%)	T_2 (35%)	T_3 (40%)	T_4 (45%)	T_5 (50%)	T_6 (FM)	p-value
Rearing period (Days)	90	90	90	90	90	90	----
Average initial weight (g)	15.21±1.49	15.87±1.16	15.29±1.43	15.38±0.81	15.81±0.96	15.38±1.41	$0.639^{n.s}$
Average final weight (g)	29.54±2.60	34.90±2.13	37.03±3.081	47.12±4.30	31.35±2.11	25.86±0.96	0.000^{***}
Live weight gain (g)	14.33±1.33	19.03±0.97	21.74±0.25	31.74±0.98	15.54±1.22	10.33±1.88	-----
Live weight gain (%)	94.21	119.91	142.18	206.37	98.29	66.51	-----
Average initial length (cm)	11.58±0.95	11.34±0.94	11.21±1.12	11.20±1.19	11.3±1.13	11.23±1.04	$0.998^{n.s}$
Average final length (cm)	16.67±1.09	17.26±0.66	17.31±1.19	17.81±0.96	16.94±1.38	16.56±1.12	0.03^{ns}
Length gain (cm)	8.94±2.16	10.13±2.02	10.7±1.62	11.6±0.84	9.2±0.28	8.6±1.36	----
Length gain (%)	77.20	89.32	95.53	103.57	81.41	76.78	----
Daily growth rate	1.046	1.332	1.579	2.293	1.092	0.739	----
Survival (%)	95.55	97.78	100.00	100.00	93.33	93.33	----
Condition factor	0.648±0.12	0.683±0.09	0.729±0.15	0.848±0.17	0.666±0.15	0.583±0.11	0.000^{***}
Feed conversion ratio	7.09	6.11	5.87	4.10	6.33	9.18	0.006^{**}
Protein efficiency ratio	0.522	0.539	0.538	0.615	0.322	0.183	0.029^{ns}
Specific Growth rate	0.320	0.380	0.427	0.540	0.330	0.246	0.01^{*}
Production Kg/m^2/90days	0.015	0.020	0.024	0.035	0.016	0.011	0.000^{***}

Table: 3 Water quality parameters of Aquaria

Water body parameters	Diet variables											
	T_1(30%CP)		T_2(35%CP)		T_3(40%CP)		T_4(45%CP)		T_5(50%CP)		T_6 (Fish Meal)	
	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range	Mean ±SD	Range
Air temp. (°C)	40.36±2.28	37.4-43.5	39.93±1.98	37.4-43.2	39.50±1.58	37.4-43.2	39.64±1.52	37.4-43.2	39.64±1.62	37.4-41.5	40.12±1.18	38.2-41.2
Water temp. (°C)	32.04±0.88	30.4-32.8	32.14±0.89	30.6-33.2	32.14±0.96	30.4-33.2	32.23±0.96	30.5-33.2	32.31±0.91	30.8-33.4	32.15±0.92	31.3-32.3
pH	7.41±0.20	7.2-7.8	7.46±0.18	7.2-7.8	7.54±0.15	7.3-7.7	7.56±0.13	7.4-7.7	7.73±0.17	7.5-7.9	7.72±0.15	7.54-7.8
Dissolved Oxygen (mg/L)	6.91±0.34	6.49-7.4	6.95±0.37	6.4-7.49	6.76±0.26	6.4-7.1	6.76±0.16	6.5-6.9	6.36±0.15	6.1-6.6	6.54±0.21	6.53-6.56
Total Alkalinity (mg/L)	155.81±7.56	142.6-164.5	155.48±5.98	148.5-165.3	155.71±4.98	146.6-160.3	158.37±2.96	153.7-162.6	162.09±1.80	159.2-164.6	158.82±1.83	157.12-158.9
Total Hardness (mg/L)	1331.1±87.03	1227.6-1453.8	1319.9±96.06	1122.6-1418.5	1325.1±95.34	1128.2-1412.8	1332.5±85.39	1144.8-1395.3	1348.1±80.22	1174.3-1412.4	1362.57±72.84	1361.35-1364.2
Nitrite (mg/L)	0.46±0.045	0.4-0.5	0.44±0.05	0.36-0.5	0.44±0.06	0.33-0.5	0.44±0.06	0.36-0.51	0.43±0.06	0.31-0.5	0.44±0.07	0.38-0.46
Nitrate (mg/L)	0.018±0.009	0.01-0.03	0.022±0.004	0.02-0.03	0.03±0.01	0.02-0.03	0.03±0.00	0.02-0.03	0.03±0.01	0.02-0.03	0.019±0.02	0.018-0.02
Total dissolved solid (ppm)	983.05±56.05	912.8-1068.3	972.14±35.30	927.4-1026.6	980.83±46.50	948.4-1076.7	970.49±15.37	957.3-995.6	1003.2±29.29	976.2-1058.5	993.02±27.81	992.04-994.56
Total ammonia (mg/L)	0.36±0.05	0.27-0.42	0.36±0.02	0.32-0.4	0.35±0.03	0.31-0.39	0.37±0.02	0.34-0.4	0.40±0.02	0.38-0.42	0.29±0.01	0.28-0.31
Total Phosphorus (mg/L)	1.55±0.26	1.22-2.02	1.53±0.14	1.34-1.75	1.54±0.06	1.43-1.62	1.49±0.05	1.42-1.57	1.49±0.09	1.33-1.59	1.49±0.13	1.39-1.52
Conductivity (mS/cm)	12.4±0.83	11.2-13.5	11.87±0.39	11.4-12.5	12.47±0.21	12.2-12.8	12.27±0.28	11.8-12.6	12.16±0.36	11.5-12.5	12.27±0.32	11.89-12.58
Salinity (ppm)	2.66±0.19	2.36-2.86	2.53±0.21	2.18-2.86	2.60±0.11	2.46-2.74	2.59±0.17	2.26-2.78	2.62±0.18	2.36-2.83	2.61±0.16	2.56-2.63

"Mean value ±Standard Deviation; Range".

Table 4. Effect of various protein diets on haematological parameters of *Sperata seenghala* reared in aquaria.

Haematological variables	Diet variables (Treatments)												
	T_1 (30%)		T_2 (35%)		T_3 (40%)		T_4 (45%)		T_5 (50%)		T_6 (Fish Meal)		
	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	Mean±S.D.	Range	p-value
WBC(10^3/µL)	169.72±0.88	168.56-170.98	127.48±1.57	125.63-129.41	124±0.63	123.11-124.85	106.64±1.38	104.76-108.34	136.62±1.30	135.31-138.53	140.36±0.73	139.37-141.39	0.000***
LYM(10^3/µL)	149.43±1.90	147.33-151.6	117.15±1.68	115.32-119.29	113.02±1.27	111.33-114.28	100.02±1.39	97.78-101.45	118.76±1.22	116.94-119.93	119.63±1.23	117.67-120.82	0.000***
MON(10^3/µL)	10.62±1.65	9.292-13.44	6.246±0.37	5.96-6.78	6.001±0.48	5.4-6.54	3.83±0.22	3.52-4.10	10.27±0.73	9.563-11.20	11.793±0.78	10.93-12.95	0.000***
GRA(10^3/µL)	9.66±3.95	5.375-13.664	4.07±0.52	3.33-4.56	4.978±0.57	4.34-5.811	2.77±0.17	2.51-2.98	7.575±1.62	5.791-8.851	8.928±0.82	8.014-9.968	0.000***
LYM (%)	88.05±1.42	86.12-89.73	91.9±0.38	91.48-92.31	91.14±0.61	90.46-91.7	93.8±0.24	93.5-94.1	86.94±1.19	85.5-88.6	85.24±0.95	83.9-86.2	0.000***
MON (%)	6.26±1.01	5.33-7.93	4.9±0.32	4.58-5.3	4.84±0.32	4.39-5.2	3.6±0.21	3.3-3.9	7.52±0.36	7.1-8.1	8.4±0.42	8.1-9.1	0.000***
GRA (%)	5.68±1.79	3.17-7.94	3.2±0.49	2.6-3.6	4.02±0.65	3.22-4.8	2.6±0.16	2.4-2.8	5.54±0.88	4.3-6.4	6.36±0.53	5.7-7	0.000***
RBC(10^6/µL)	2.128±0.13	1.95-2.25	1.96±0.08	1.89-2.11	1.75±0.06	1.69-1.82	1.69±0.13	1.54-1.87	2.358±0.13	2.15-2.51	2.39±0.09	2.26-2.51	0.000***
HGB(g/dl)	6.698±0.35	6.36-7.12	6.92±0.20	6.69-7.17	7.5±0.35	6.98-7.82	8.25±0.21	7.98-8.48	6.618±0.41	6.09-7.14	6.62±0.20	6.4-6.9	0.000***
HCT (%)	39.1±.1.17	37.5-40.4	35.32±0.59	34.7-36.3	36.2±1.22	34.4-37.8	27.82±1.07	26.74-29.6	41.32±0.86	40.3-42.5	41.98±1.15	40.4-43.5	0.000***
MCV(µm³)	171.22±4.55	165.3-177.8	176.8±5.49	168.1-183.2	195.06±17.31	164.7-208.3	177.78±5.46	169.7-184.9	174.48±4.68	169.3-182.5	174.12±3.41	169.3-178.3	0.000***
MCH(pg)	38.02±1.17	36.6-39.3	36.04±1.21	34.4-37.2	39.36±1.99	36.5-41.6	39.6±2.39	36.3-42.6	36.7±0.65	35.88-37.41	37.62±0.88	36.2-38.5	0.0001***
MCHC(g/dl)	22.266±0.79	21.13-23.1	19.24±0.94	18.13-20.28	20.3±1.50	18.6-22.3	23.54±1.23	21.9-25.2	21.28±0.23	21-21.53	22.1±0.38	21.7-22.68	0.000***
RDW (%)	9.62±0.21	9.4-9.9	10.52±0.25	10.2-10.9	11.02±0.46	10.5-11.45	10.32±0.28	9.9-10.7	9.9±0.315	9.5-10.3	9.78±0.26	9.5-10.2	0.000***
RDW-SD(µm³)	79.632±1.99	76.95-82.21	83.8±3.48	79.2-88.9	175.98±8.13	161.8-182.6	88.08±4.17	81.87-93.2	80±0.99	78.7-81.3	80.14±1.22	78.5-81.7	0.000***
PLT(10^3/µL)	186.52±5.26	178.63-191.79	120.28±1.74	117.9-122.5	166.3±5.24	157.2-170.2	176.5±5.49	168.2-183.2	191.3±3.48	185.8-194.67	192.84±2.10	190.83-195.8	0.000***
MPV(µm³)	6.34±0.19	6.1-6.6	6.56±0.196	6.3-6.78	5.94±0.20	5.78-6.3	6.08±0.23	5.77-6.3	6.26±0.94	6.1-6.32	6.26±0.14	6.1-6.4	0.0007***
PCT (%)	0.169±0.014	0.153-0.189	0.076±0.02	0.03-0.09	0.09±0.004	0.086-0.098	0.107±0.003	0.103-0.112	0.112±0.008	0.098-0.119	0.119±0.005	0.113-0.127	0.000***
PDW (%)	9.174±0.34	8.74-9.54	10.92±0.23	10.7-11.2	8.94±0.11	8.81-9.1	10.28±0.46	9.8-10.9	8.96±0.169	8.78-9.16	9.082±0.183	8.87-9.27	0.000***

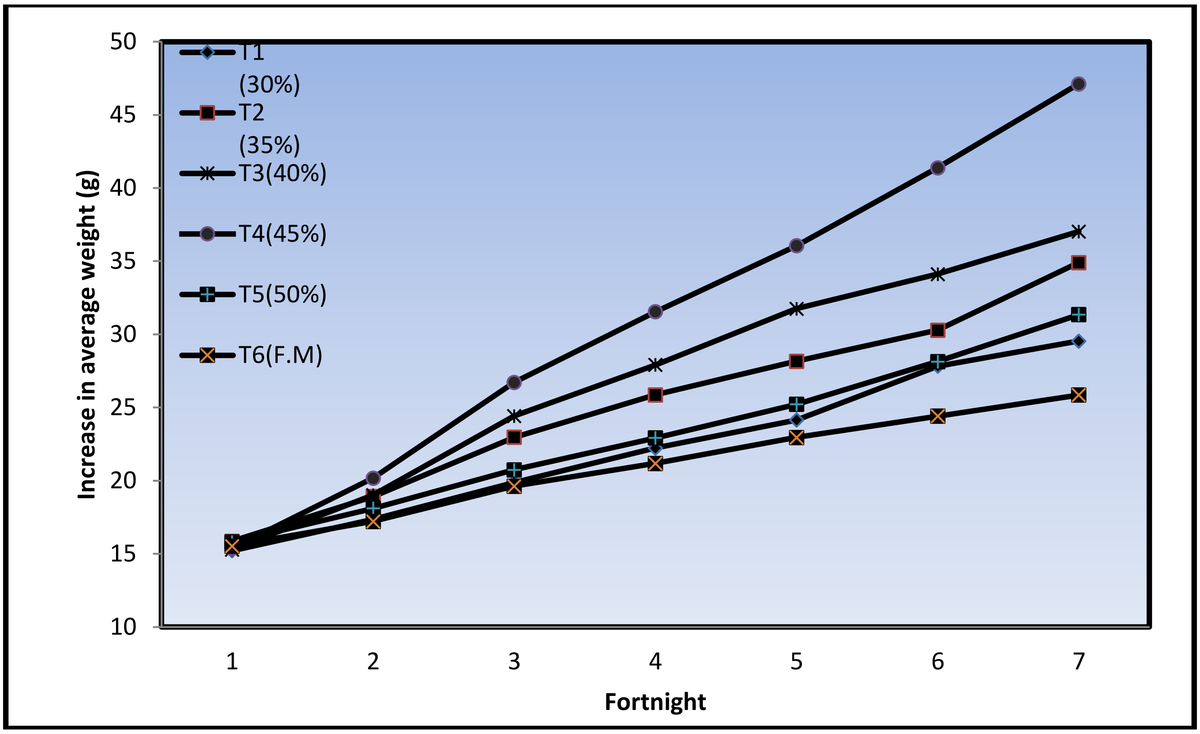

Fig.1: Effect of nutritional diets on fortnightly increase in average weight of *Sperata seenghala* reared in aquaria at various protein diets.

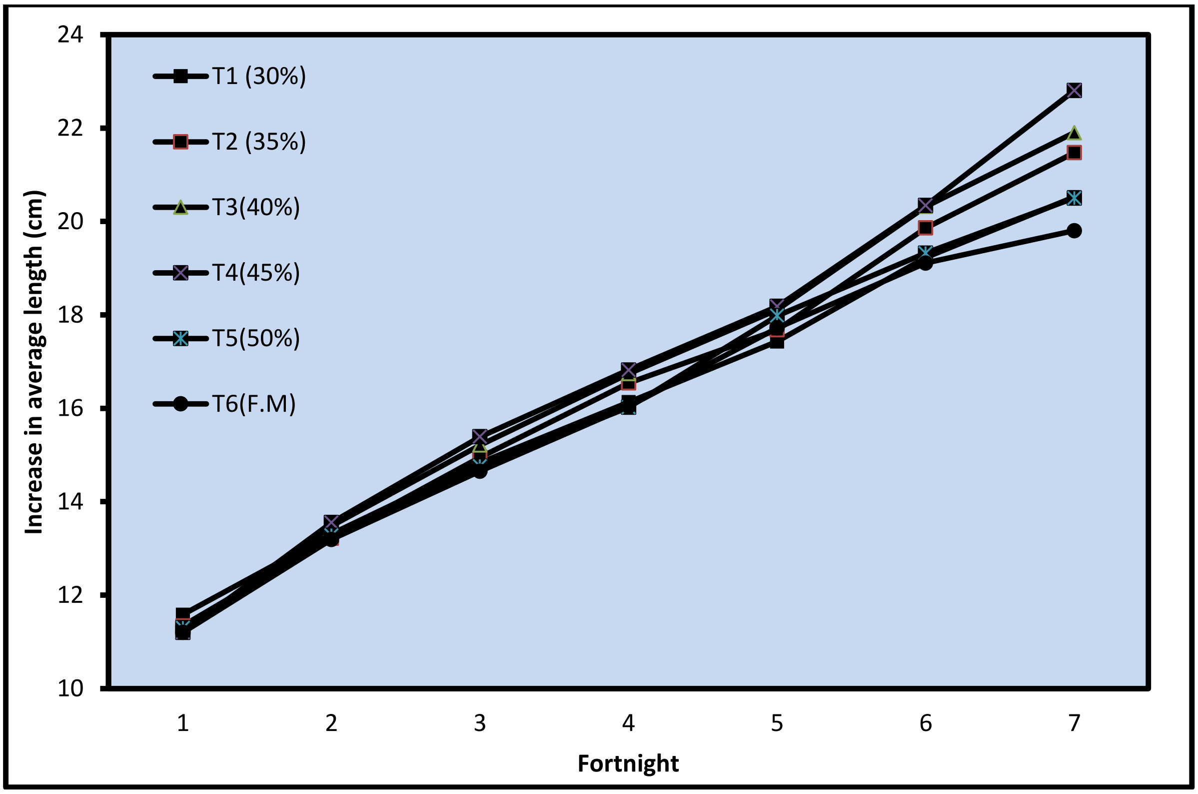

Fig.2: Effect of nutritional diets on fortnightly increase in average length of *Sperata seenghala* reared in aquaria at various protein diets.