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Effect of Different Types of Plants (*Lemna* Sp., *Azolla filiculoides* and *Alfalfa*) and Artificial Diet (With Two Protein Levels) on Growth Performance, Survival Rate, Biochemical Parameters and Body Composition of Grass Carp (*Ctenopharyngodon idella*)

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Abstract

A growth trial experiment was conducted for 90 days to evaluate between two aquatic plants (*Lemna* sp. and *Azolla filiculoides*), a xerophilous plant (*Alfalfa*) and two formulated diets with different protein levels (25 and 35%) on growth performance, survival rate, biochemical parameters and body composition of grass carp (*Ctenopharyngodon idella*). Initial weight of grass carp was 15.41 ± 0.51 g in five treatments with three replicates in each treatment experiments under field and fiberglass condition. The grass carp in experimental treatments were fed by Fed plant origin diet with 20 percent of body weight and formulated diet with 5 percent of body weight (3 times a day). Growth performance, survival rate, Feed Conversion Ratio (FCR), Specific Growth Rate (SGR), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), hematocirit, hemoglobin and total protein were found higher for alfalfa diet group compared to all other experimental diets ($P<0.05$). These results suggest that alfalfa diet appears to be more adequate for a better growth of this fish, followed by *Lemna* sp. and had significant difference from other treatments ($P<0.05$). The lowest growth performance, survival rate and biochemical parameters measurements was observed in pellet diet containing 25 percent protein that it had significant difference to other treatments ($P>0.05$). And also in growth parameters, *A. filiculoides* and formulated feed with 35% protein was not significantly different from each other ($P<0.05$). In blood factors, there was not observed any significant differences in Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBCs), red blood corpuscular (RBCs), glucose, cholesterol and chortisol ($P<0.05$) between treatments. Also in body composition the highest lipid was observed in group fed with pelleted diet (25% percent protein) however, it had not significant difference to group fed with pelleted diet (35% percent protein) ($P<0.05$) and all treatments did not show any significant differences in moisture, protein and ash measurements among each other ($P<0.05$).

Keywords: *Ctenopharyngodon idella*; *Alfalfa; Lemna* Sp.; *Azolla filiculoides*; Formulate diet; Growth performance; Biochemical parameters; Body composition

Introduction

The grass carp is a rapid growing, phytophagous, cyprinid fish indigenous to the large rivers of China and Siberia [1]. The ability to turn large quantities of a wide variety of plant material into good quality protein has made this species an important aquaculture candidate worldwide [2].

In recent years a number of investigations were conducted on fish Polyculture in world using animal wastes to supplement pelleted fish feed, thereby reducing fish production costs, Lately, investigations were carried out on the integration of fish and vegetable production, and a duck-fish vegetable integrated system [3-6]. Under suitable conditions, adult grass carp will eat more than its own weight of plant material on a daily basis. However, the grass carp is not an exclusively herbivorous fish species, as it also needs food of animal origin [7].

Good nutrition in animal production stems is essential to economically produce a healthy, high quality product. Fish nutrition has advanced apically in recent years with the development of new balanced commercial diets that promote optimal fish growth and health. The development of new species-specific diet formulations supports the aquaculture (fish farming) industry as it expands to satisfy increasing demand for affordable, safe and high-quality fish products. Even when the natural feed forms the main source of nutrition, supplemental feeding with artificial feed is necessary to obtain increased production in ponds [8]. Although the use of artificial feeds may be costly, especially the commercially compounded ration, they have advantage in fish culture:

1: Enable high stocking density (stocking maximization) especially in polyculture system.

2: They promote faster growth of fish, since food will always be available.

3: High fish yield is guaranteed relative to the stocking density.

4: Uneaten artificial feeds in the pond water will be biologically degraded. This acts as fertilizer to promote plankton growth.

5: A fish farmer while feeding the fish can study the behavior of fish and monitor their health.

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The optimization of fish production requires research into feeding techniques, which promotes growth and at the same time reduces the quantity of waste products released in the water as reported by Singh et al. [9] According to Erondu et al. [10] and Sheunn et al. [11], fish feed consist of 60% production cost and the protein component is to be the most expensive in terms of overall feed cost. Increasing protein levels in feeds can lead to improved fish production, but excessive dietary protein is not economical for fish culture. The dietary protein level is one of the major factors influencing growth of fish, feed efficiency and water quality. The protein requirement of fish is recognized as the protein content which gives the maximum growth, maximum economic profit and maximum protein deposition [12,13]. According to Ajiboye and Yakubu [14] the determination of protein requirement of fish is a very critical factor in aquaculture production. Abidi and Mukhtar [15] emphasize the need to minimize protein levels of the fish, which reduces the proportion of dietary protein that is metabolized, without reducing growth, resulting in undesirable nitrogenous waste production. According to Engin and Carter [16]; Jensen [17] and Kalla et al. [18], determination of the required dietary protein levels is important to get highest growth and reduce the water deterioration problems related with supplementary feed intake of fish. Although toxicity of metabolites in fish varies considerably and depends on many internal and external factors but amount of nitrite, ammonia, and orthophosphate produced by the fish seems to be directly related to the source and levels of protein intake.

The objective of the present study was to determine the effect of plant feed and formulated feed on growth performance survival rate, biochemical parameters, body composition in grass carp.

Materials and Methods

Fish and laboratory condition

Before starting the experiments water was dechlorinated. The animals were kept in fiberglass tank under controlled conditions (14 h light: 10 h darkness). 150 uniform grass carp with initial weight: 15.41 ± 0.51 g were obtained from the Institute of Pond Fish Culture in Sari, Iran. Five treatments with three replicates for each one experiments under field and fiberglass conditions (each with a capacity of 450 liters). The densities of fish per tank were 10 fish. And eight fiberglass tanks were for replacement water. Water quality parameters of input water for rearing system were monitored each week throughout the experimental period. The water temperature was 19.46 ± 1.23°C, pH was 7.85 ± 0.26 and water oxygen level was maintained above 7.65 ± 0.55 mg L^{-1} during the experiment an electrical air pump (by a single filtration unit). The fish were weighed individually at the beginning and at the end of the experiment. Before distributing fish to the experimental tanks (in the beginning of exogenous feeding), 50 fish were sampled from the holding tank for biometry. At the end of the experiment, all fish from each tank were sampled and the final weight and length of body were measured. Growth parameters of fish were calculated based on the data of biometry of grass carp.

Diet

Formulated diet was provided by aquatic foods company sijaval Gorgan, Iran. Nutrient compositions of experimental diets are given in table 1.

A. filiculoides and *Lemna* sp. were collected from lake in Gorgan, Iran. Every three days and hold they in water tank in laboratory. Also Alfalfa was collected from a farm near the city every three days and hold in refrigerator.

Ingredients	*Lemnea* sp.	*Azolla Filiculoides*	*Alfalfa*	Formulated Pellet with 35% protein	Formulated Pellet with 25% protein
Protein (%)	28	31	50.8	35	25
Lipid (%)	11.4	9.9	10.2	13	14
Fiber (%)	2.7	2.8	14.4	18	18
Ash (%)	6	4.1	8	11	11

Table 1: Nutrient composition of experimental diets (%).

Feed analyze

Proximate composition of diets was carried out using the Association of Analytical Chemists [19] methods. Protein was determined by measuring nitrogen (N×6.25) using the Kjeldahl method; Crude fat was determined using petroleum ether (40–60 Bp) extraction method with Soxhlet apparatus and ash by combustion at 550°C.

Feeding Rate

The grass carp in experimental treatments were fed of *Lemna* sp. diet, *Azolla filiculoides* diet and *Alfalfa* diet with 20 percent of their body weight and formulated diet (with different protein 25 and 35%) were fed with 5 percent of their body weight for 3 times a day (6.00, 14.00 and 22.00).

Sampling and analytical methods

To reduce stress by a hammer butt to the head of fish and cut off the tail of fishes and samples were collected from tail to eppendorff, and sent to laboratory on ice. Blood was placed in non-heparinized tubes and left to clot at 4°C for 15 min., Afterwards, tubes were centrifuged at 3000 rpm using an Eppendorff centrifuge for 10 min to obtain serum. The sera were separated into aliquots and were frozen and stored at -80°C until metabolite analyses. All samples were immediately immersed in liquid nitrogen and then transferred to -80°C freezer until analysis. The quantitative determination of serum glucose was carried out using commercially available diagnostic Experimental Protocols kits Pars Azmoon, Iran (1 500 0178), at 546 nm and 37°C by the glucose oxidase method according to Trinder [20]. Serum total protein levels were determined using Pars Azmoon, Iran (1 500 028) kit, with bovine serum albumin serving as standard at 546 nm and 37°C.

Analytical methods for body composition

At the end of the feeding period (90 days), tissue samples were taken from randomly selected fish. Following overnight fasting, after the fish were killed by blunt trauma to the head, samples of muscle were rapidly excised, frozen and stored at -20°C until body composition analysis. The whole fish and diet samples were ground and then analyzed for dry matter after desiccation in an oven (105°C for 24 h), ash (550°C, overnight), crude protein (6.25×nitrogen (N); Kjeltec Auto System), lipid (Soxtec HT6 after hydrolysis with HCl).

Determination of growth parameters

Growth parameters were calculated as follows: final body weight (WG)=final body weight (g)–initial body weight (g). Specific growth rate (SGR) (%BW day^{-1})=(Ln final body weight (g)-Ln initial weight (g))/(experimental period)×100. Feed conversion ratio (FCR) (%)=(total fed/body weight increase (g))×100. Daily growth rate (DGR)=(final body weight (g)–initial weight (g))×(100)/(experimental period×initial weight (g)).

Statistical analysis

In order to determine significant differences, results were analyzed

Treatments Growth Indices	T1 Fed with *Lemna* Sp	T2 Fed with *Azolla filiculoides*	T3 Fed with *Alfalfa*	T4 Supplemented Pellet with 35% protein	T5 Supplemented Pellet with 25% protein
Initial weight (g)	15.35 ± 0.27^{a}	15.81 ± 0.33^{a}	15.77 ± 0.23^{a}	15.31 ± 0.62^{a}	14.79 ± 0.43^{a}
Final body weight (g)	25.31 ± 0.37^{b}	20.85 ± 0.84^{c}	45.61 ± 0.95^{a}	20.68 ± 0.45^{c}	16.71 ± 0.27^{d}
Body weight increased (g)	9.96 ± 0.1^{b}	5.04 ± 0.53^{c}	29.83 ± 0.75^{a}	5.37 ± 0.83^{c}	1.92 ± 0.44^{d}
Initial length (cm)	11.53 ± 0.21^{a}	11.3 ± 0.11^{a}	11.4 ± 0.1^{a}	11.2 ± 0.2^{a}	11.3 ± 0.36^{a}
Final length (cm)	15.7 ± 0.11^{b}	14.4 ± 0.0^{c}	17.2 ± 0.44^{a}	14.43 ± 0.58^{c}	12.7 ± 0.17^{d}
Body length increased (cm)	4.2 ± 0.1^{b}	3.07 ± 0.11^{c}	5.8 ± 0.35^{a}	3.23 ± 0.25^{c}	1.4 ± 0.26^{d}
Specific growth rate for weight (% BWday^{-1})	0.55 ± 0.0^{b}	0.31 ± 0.02^{c}	1.18 ± 0.01^{a}	0.33 ± 0.05^{c}	0.14 ± 0.03^{d}
Feed Conversion Ratio (%)	35.29 ± 0.59^{b}	62.18 ± 4.29^{a}	15.61 ± 0.25^{c}	15.3 ± 0.09^{c}	37.2 ± 2.78^{b}
Feed Conversion efficiency (%)	0.3 ± 0.01^{b}	0.2 ± 0.01^{b}	0.6 ± 0.0^{a}	0.7 ± 0.0^{a}	0.02 ± 0.01^{b}
Condition Factor	0.65 ± 0.01^{b}	0.7 ± 0.03^{c}	0.9 ± 0.06^{a}	0.69 ± 0.01^{b}	0.82 ± 0.02^{d}
Daily growth ratio (g)	0.11 ± 0.0^{b}	0.06 ± 0.01^{c}	0.33 ± 0.01^{a}	0.06 ± 0.01^{c}	0.02 ± 0.0^{d}
Survival rate (%)	94.28 ± 4.28^{a}	94.28 ± 4.28^{a}	98.09 ± 2.18^{a}	94.28 ± 4.28^{a}	79.99 ± 5.71^{b}

Groups with different alphabetic superscripts at the same row differ significantly at P<0.05 (ANOVA)

Table 2: Growth parameters and survival rate of Grass carp (*Ctenopharyngodon idella*) in experimental treatments (trial 1-5).

Treatments Blood index	*Lemna* Sp.	*Azolla filiculoides*	Alfalfa	supplemented Pellet with 35% protein	supplemented Pellet with 25% protein
WBC (×10/mm^{3})	7.5 ± 1/1	7.4 ± 1.9	7.2 ± 1.2	7 ± 2.9	7.3 ± 1.5
RBC (×10/mm^{3})	1.84 ± 0.06	1.83 ± 0.12	1.86 ± 0.03	1.81 ± 0.2	1.74 ± 0.01
Hemoglobin (g/dl)	8.83 ± 1.3^{a}	8.66 ± 1.21^{a}	9.1 ± 1.17^{a}	7.5 ± 1.3^{b}	7.1 ± 1.17^{b}
Hematocrit (%)	25.7 ± 3.56^{ab}	25.2 ± 3.01^{ab}	29.82 ± 2.15^{a}	24.9 ± 2.29^{ab}	20.11 ± 2.21^{b}
MCV(fl)	144.21 ± 8.88b^{c}	135.35 ± 7.4^{c}	156.47 ± 11.29^{a}	135.53 ± 8.42^{c}	137.33 ± 15.61^{c}
MCH (pg)	45.89 ± 3.15b^{c}	41.85 ± 2.77^{c}	49.87 ± 3.21^{a}	41.71 ± 2.64^{c}	41.51 ± 3.08^{c}
MCHC (%)	32 ± 24 ± 7.65	31.71 ± 6.52	31.98 ± 8.48	31.11 ± 11.21	30.74 ± 12.46

Groups with different alphabetic superscripts at the same row differ significantly at P<0.05 (ANOVA)

Table 3: Biochemical parameters of Grass carp (*Ctenopharyngodon idella*) in experimental treatments (trial 1-5).

by one-way Analysis of variance (ANOVA) and Duncan's multiple range tests were used to analyze the significance of the difference among the means of treatments by using the SPSS program.

Results

Growth performance

Results clearly showed specimens that were fed with *Alfalfa* had positive effects on the growth parameters and had significant difference from other treatments (P<0.05).

The maximum final body weight (FBW) in treatments (Table 2) was fed on Alfalfa (45.61 ± 0.95 g), followed by *Lemna* sp. (25.31 ± 0.37 g) and had significant difference from other treatments (P<0.05). *A. filiculoides* (20.85 ± 0.84 g) and formulated meal with 35% protein (20.68 ± 0.45 g) was not significantly different from each other (P<0.05). The lowest final body weight of specimens in treatments was on formulated diet with 25% protein (16.71 ± 0.27) (P<0.05).

The highest specific growth rate (SGR) for weight was observed *Alfalfa* meal (1.18 ± 0.01%) and had significant difference from other treatments (P<0.05). followed by *Lemna* sp. (0.55 ± 0.0), *A. filiculoides* (0.31 ± 0.02%) and formulate meal with 35% protein (0.33 ± 0.05%) was not significantly different from each other (P<0.05). The lowest SGR of treatments was on formulated diet with 25% protein meal (0.14 ± 0.03) (P<0.05).

This is particularly true for survival rate, where the highest was obtained in the experimental treatment Alfalfa. The highest survival rate observed on *Alfalfa* meal (98.09 ± 2.18%), followed by *Lemna* sp., *A. filiculoides* and formulated meal with 35% protein (94.28 ± 4.28%) there was no significant difference among this three treatments (P<0.05). And the lowest survival rate of treatments was on formulated diet with low protein meal (25% protein) (79.99 ± 5.71%) (P<0.05).

The highest food conversion rate (FCR) was observed in AZOLLA (*A. filiculoids*) (62.18 ± 4.29) which had significant difference from other treatments (P<0.05). After that it was on *Lemna* sp. (35.29 ± 0.59%) and *A. filiculoides* (37.2 ± 2.78%) and there were no significant difference between each other (P<0.05) but they were significantly different to other treatments (P<0.05).

The lowest FCR observed in *Alfalfa* treatment (15.61 ± 0.25%) and formulated meal with 35% protein (15.3 ± 0.09%). They had no significant difference with each other (P<0.05) but they had significant difference from other treatments (P<0.05).

Biochemical parameters

The biochemical parameters and serum levels of measured parameters, in grass carp are shown (mean ± SEM) in tables 3 and 4. There were no significantly different in WBC, RBC and MCHC value between treatments (P<0.05). The highest biochemical parameters of hemoglobin was obtained in *Alfalfa* treatment and it had not any significantly different to *Lemna* sp., and *A. filiculoides* (P<0.05) and the lowest was observed in Pellet diet with 25% protein. However, this treatment had not significantly different to Pellet diet with 35% protein (P<0.05) in this comparison. The maximum of hematocrit was obtained in Alfalfa treatment but it had just significantly different to Pellet diet with 25% protein (P<0.05). The highest of MCV value was obtained in *Alfalfa* treatment followed by *Lemna* sp., but the *Lemna* sp., treatment

Treatments Serum index	*Lemna* Sp.	*Azolla filiculoides*	Alfalfa	supplemented Pellet with 35% protein	supplemented Pellet with 25% protein
Albumin (g/dl)	1.51 ± 0.08^{ab}	1.42 ± 0.06^{ab}	1.85 ± 0.05^{a}	1.33 ± 0.06^{b}	1.23 ± 0.01^{b}
Globulin (g/dl)	2.17 ± 0.4^{ab}	2.03 ± 0.2^{ab}	2.25 ± 0.1^{a}	2.32 ± 0.1^{a}	1.9 ± 0.3^{b}
Glucose (mg/dl)	80.28 ± 12.25	81.33 ± 12.25	83 ± 6.21	88.22 ± 4.66	89.42 ± 8.11
Cholesterol (mg/dl)	206.18 ± 35.45	202.67 ± 31.18	201.33 ± 40.25	221 ± 35.51	211 ± 31.32
Total Protein (g/dl)	3.68 ± 0.7^{ab}	3.45 ± 0.8^{ab}	4.1 ± 0.8^{a}	3.65 ± 0.9^{ab}	3.13 ± 0.9^{b}
Cortisol (µg/dl)	29.17 ± 13.12	28.27 ± 9.62	27.2 ± 12.06	31.2 ± 7.09	30.3 ± 8.85

Groups with different alphabetic superscripts at the same row differ significantly at P<0.05 (ANOVA)

Table 4: Serum levels of Grass carp (*Ctenopharyngodon idella*) in experimental treatments (trial 1-5).

Treatments Index	*Lemna* Sp.	*Azolla filiculoides*	Alfalfa	supplemented Pellet with 35% protein	supplemented Pellet with 25% protein
Moisture	78.5 ± 23.9	78.4 ± 20.9	78.87 ± 17.2	74.16 ± 22.2	75.42 ± 21.2
Protein	14.12 ± 3.9	14.62 ± 4.12	14.96 ± 3.6	14.88 ± 4.22	14.1 ± 4.14
Lipid	4.7 ± 1.37b	4.8 ± 1.21b	4.12 ± 1.17b	7.21 ± 2.28a	7.47 ± 1.78a
Ash	2.71 ± 0.86	2.19 ± 0.61	2.8 ± 0.95	2.79 ± 1.23	3.1 ± 1.23

Groups with different alphabetic superscripts at the same row differ significantly at P<0.05 (ANOVA)

Table 5: Body Composition of Grass carp (*Ctenopharyngodon idella*) in experimental treatments (trial 1-5).

had not any significantly different to *A. filiculoides* and pellets diet (P<0.05).

Serum levels

In the serum levels of glucose, cortisol and cholesterol value had not any significant differences between Plant and artificial diet in present study (Table 4).

The maximum of total protein was obtained in *Alfalfa* treatment. However, this treatment had not significantly different to *Lemna* sp., (P<0.05). The lowest of total protein was observed in Pellet diet with 25% protein and it had not significantly different to *A. filiculoides, Lemna* sp., and Pellet diet with 25% protein (P<0.05).

Body Composition

The result of body Composition is present in table 5. According the result there were not any significantly different in moisture, protein and ash content between the experimental treatment (P<0.05).

At the end of the experiment, the lipid content in fish fed artificial died was significantly higher than those fish fed plan diet (P<0.05) and in Pellet diet with less protein pellet was higher than high protein pellet diet. However, it was not significantly difference (P<0.05).

Discussion

The result of body composition showed that using of formulated diet had not significantly affect quality of whole body values like protein, moisture and ash. However, the highest protein content was obtained in *Alfalfa* treatment. But fed formulated diet showed significant increased in lipid value. Yudu [21] found formulated diet with 23 and 35% protein had not any significantly different on protein content whole body in juvenile grass carp. This result is in agreement with the results obtained during present study. Also reported the formulated diet with different protein levels (25&35%) had not any significantly different on lipid value, this result is not agreement with our finding. Hemoglobin value with age and condition will be changed. This value for some species reported: grass carp is 7-9 g/dl [22], *Essex lusicus* 5.6-15 g/dl [23], *Oncorhynchus mykiss* 8.8-9.6 g/dl [24], *Cirrhina mrigala* 7.6-11.8 g/dl [25].

Study on last research showed biochemical parameters and serum levels affected with species [22], age [26], water temperature [27] and diet [28]. In the present study different type of plant and formulate diet had not any significantly different most of the biochemical parameters and serum levels that is showed the formulate diet can compete to Fed plant origin diet and replacement instead Fed plant origin diet.

Some studies seem to indicate that the type and rate of fish consumption, and its growth, are related to the chemical content or nutritive value of the plants, such as the gross energy content of the diet and the dietary protein [29,30].

Filizadeh et al. [31] found that smaller fish had selected softer plant tissue and youngest plants, while bigger fish eat a wide variety of tough and fibrous plants. *Lemna* sp, had shown as preferred whilst *A. filiculoides*, are generally avoided. This finding agrees with our results.

Catrino et al. [32] found two soft tissue, *A. filiculoides* and *Lemna* sp., were cleary preferred by grass carp, whilst other species such as *potamogeton pectinatus* and *Myrophyllum spicatum* were moderately eaten. These results disagree with our findings, because *Lemna* sp. is eaten better than *A. filiculoides* and performed better growth parameters. However, in survival rate they were not significantly different to each other (p<0.05).

Most of other species, gastrointestinal evacuation time normally exceed 24h [33-35]. The intestinal evacuation time of grass carp (12h) is much shorter than other fish. However, most of those studied fish are carnivorous or omnivorous, and also have well-developed stomach. In natural environment, the prey of these carnivorous or omnivorous fish consistent of small fishes, shrimps and other aquatic animals [36]. However, grass carp is herbivorous and stomachless. Compared to the prey other carnivorous or omnivorous fish, the natural food of grass carp is low-energy and low protein content, so it should be very necessary to improve the efficiency of digestion and absorption to shorten the passing time of food in intestine, for obtaining enough energy and protein. This hypothesis is supported by several evidences: 1) grass carp have much longer intestine. 2.29-2.54 times to its body length, than carnivorous fish [37]. The long intestine could enlarge the surface area of digestion and absorption and it also could lead to shorten the evacuation time. 2) Lin [37] found in a single daytime, the time used of grass carp for eating is about 20h and it only stops eating in midnight for 4 h in natural environment. It proves that the ability of

digestion in grass carp was strong. Li et al. [38] also found when grass carp was fed phytoplankton and vegetable to satiety, it only needed 4h to evacuate all the content in intestine. Jobling [39] believed the evacuation of high energy density artificial diet was slower than prey in natural environment, which was also confirmed by the present study which demonstrated that the intestinal evacuation time of formulated diet (12h) is 3 times longer than natural food of grass carp.

Therefore, making a proper diet is essential to improve the efficiency of digestion and absorption, which this in turn causes getting enough energy and protein. Thus, in this research we have used two diets with different protein percentage, and it was clearly observed that treatment (case) fed in a diet with high protein (35%) had better growth performance and less food conversion ratio (FCR) than the other case under a diet with low protein (25%). On the other hand, treatment fed *A. filiculoides* was not significantly different from treatment fed contain 35% protein ($P<0.05$). Also the highest FCR was obtained in formulate diet with low protein similar result reported by Ghazala et al. [40] Hossain et al. [41] and Gao et al. [42].

According to this study, one of the most desirable plant foods for grass carp is *Alfalfa* and one of the most desirable artificial foods is pellets with high protein that had acceptable growth. However, growth response of fish varies from species to species due to difference in feeding habit, water temperature and fish size. Gao et al. [43] reported improved growth in grass carp when dietary protein level increased from 25% to 38%. This result agrees with our finding. However, in another study Gao et al. [43] reported 39% optimum protein level in grass carp. In this study, the approximate level of dietary protein, which showed maximum growth in grass carp (*Ctenopharyngodon idella*) was found to be 35%. Similar results for growth indices were observed by Jana et al. [44] for *Chanos chanos*; Ogunji and Wirth [45] for *Oreochromis niloticus* and Stapathy et al. [46] for *Labeo rohita*. Sa et al. [47] reported that unbalanced diet do not fulfill the basic requirements of animals. In present study lowest growth in fish fed 25% protein diet (PL) may be due to the reduced feed intake of the diet with a protein level lower than that required for body repairing and maintenance. Similar findings were reported by Sa et al. [47-49]. Nekoubin and Sudagar [50] reported that formulate diet had significantly effect on growth performance and survival rate to comparison with *Phragmites communis* in juvenile grass carp. Also Nekoubin and Sudagar [51] reported supplemented pellet contain 25% protein with synbiotc had not positive affect in growth performance in comparison with pellet contain 35% protein in grass carp.

Hossain et al. [41] reported that Body weight increased (BWG) and Daily growth rate (DGR) in *Tor putitora* had maximum value for high protein level fed diet and minimum value for low protein level fed diet this result is agreement with the results obtained during present studies.

Due to the excessive feed and high food conversion ratio (FCR), limitations and the lack plant for feeding this fish, food pellets containing high protein (35%) can be used as a meal replacement in mass rearing.

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