E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2017; 5(5): 278-282

www.fisheriesjournal.com
Received: 20-07-2017
Accepted: 21-08-2017

Sadar Aslam
Department of Animal Sciences, Fisheries and Aquaculture Lab, Quaid-i-Azam University, Islamabad, Pakistan

Amina Zuberi
Department of Animal Sciences, Fisheries and Aquaculture Lab, Quaid-i-Azam University, Islamabad, Pakistan

Correspondence
Sadar Aslam
Department of Animal Sciences, Fisheries and Aquaculture Lab, Quaid-i-Azam University, Islamabad, Pakistan

Effect of duckweed by replacing soybean in fish feed on growth performance of Grass carp (*Ctenopharyngodon idella*) and Silver carp (*Hypophthalmichthys molitrix*)

Sadar Aslam and Amina Zuberi

Abstract
A 90-day feeding trial was carried out to determine and compare the effects of duckweed (*Lemna minor*) and soybean (*Glycine max*) meals as a source of protein on the growth performance, growth hormone levels and proximate composition of Grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844) and Silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) in monoculture as well as polyculture system. In the present study, 35% protein practical diets, F_{SBM} (feed containing 21% soybean meal) and F_{DW} (feed having 21% duckweed) were prepared as a source of protein. At the end of the experiment, Grass carp showed a non-significantly higher growth rate as compared to Silver carp when fed F_{DW} while Silver carp showed a higher growth rate in response to F_{SBM} diet in both culture systems. A similar trend was observed in Percentage weight gain (% WG), specific growth rate (SGR%) and feed conversion efficiency (FCE%). The Grass carp and Silver carp fed with F_{DW} and F_{SBM} diets showed significantly (P<0.05) higher growth hormone levels in polyculture as compare to monoculture system. In this study, proximate composition analysis showed approximately similar composition of both diets. It can be concluded from this study, that duckweed can be used as a protein source in fish feed for the selected fish species.

Keywords: Grass carp, silver carp, duckweed, soybean meal, growth performance

1. Introduction
In a world where millions of people, especially in Asia and Africa suffer from chronic malnutrition, aquaculture will play a significant role to meet the huge challenge of global food security and economic growth [1]. The increasing aquaculture has led to dependence on artificial feeds. Protein is the costly component in fish feeds and also a very essential factor affecting growth performance of fish and feed cost [2]. Fishes have high dietary protein requirement. Reducing the costs of fish feed will be key factor for successful development of aquaculture [3]. Literature from text books shows a lot of difference in protein requirement of fish with special reference to species, age, culture system and overall ecological perspectives [4-6].
Duckweed (*Lemna minor*) is a natural protein source which has high quality of essential amino acids than most other plant proteins and shows more close resembles to animal proteins [7-11]. The duckweed contains high protein content, about 400 g/kg, closely resembles soybean meal and it has also balanced amino acid profile, particularly lysine (6.9 g/100 g protein) which is a limiting amino acid in other plant proteins [12]. Duckweed leaves contain very low amount of fiber, therefore even monogastric animals can digest it and many fishes, especially herbivorous consume duckweed readily because the cell wall of this plant has low lignin [13]. Thus, duckweed shows enhanced digestibility and is considered as an ideal protein source of fish feed [14-16]. Duckweed grown on nutrient-rich medium has a high concentration of important trace elements such as, phosphorus and potassium. It also contains pigments especially carotene and xanthophyll, which make duckweed important dietary supplement for fish feed [17].
Soybean meal (*Glycine max*) is one of the common ingredient in fish feed as it contains high protein content and adequate amino acid profile. However, it has many anti-nutritional factors such as; trypsin inhibitor, phytic acid, oligosaccharides (raffinose, stachyose), antigenic factors, lectins, saponins, lipoxidase, phytoestrogens, and goitrogens which limit its use in fish feed [18]. The reduced growth with soybean might be due to its sub optimal amino acid balance and also due to the presence of anti-nutritional factor, especially trypsin inhibitor [19].

Carps being cultured widely in many parts of the world including Pakistan [20]. Different carps having different feeding habits are reared in polyculture system in the same water bodies. The polyculture usually shows higher production rate and higher economic and financial profitability because natural productivity can be fully utilized hence the monoculture system is less frequently practiced, but in many countries, monoculture systems are still practiced because aquaculturists preferred fish species which shows higher production and survival rate when reared independently [21].

An increase in growth rate shows the higher secretion of growth hormone, a polypeptide hormone which is secreted from anterior pituitary gland. In fish, growth hormone takes part in important physiological processes in the body including the protein, lipid and carbohydrate metabolism, skeletal and soft tissue growth, regulation of ionic and osmotic balance, reproduction and immune function. Recently reported studies have suggested that growth hormone also affects several aspects of behavior such as; appetite, foraging, and predator avoidance [22, 23].

This study was designed to test the hypothesis that, duckweed as a component of a fish feed in a monoculture and polyculture system could be more economical supplement than soybean in fish diet. Therefore, the objectives of this study were: (i) to evaluate the growth performance of Silver carp and Grass carp and (ii) to determine growth hormone levels of selected fish species under monoculture and polyculture system by replacing soybean meal with duckweed in fish feed as a cheap source of crude protein.

Materials and Methods

Collection of Fish Samples

Fingerlings of Silver carp and Grass carp (of the same body weight 5.54 g ± 0.02 and length 7.18 cm ± 0.01) were purchased from Attock and Rawal fish Hatchery Islamabad respectively and transported in polythene bags filled with oxygen to Fisheries and Aquaculture laboratory, Department of Animal Sciences, Quaid-i-Azam University, Islamabad. The fishes were then acclimatized for about 15 days in fiber circular tank containing well oxygenated water before starting the experiment. The water of the tank was changed daily with dechlorinated water. Dead fishes were removed with the help of hand net quickly to avoid water fouling. During the acclimatization period temperature was maintained at 26°C, pH was 7.8, DO was 5.0 mg/L and ammonia was < 0.25 ppm. The experiment was conducted in triplicate for a period of 90 days in transparent glass tanks of dimensions (60× 30 ×30 cm) for monoculture and (90 × 45 × 45 cm) for polyculture. The selected fishes were stocked at stocking density of about 2.5 g L^{-1} in each transparent glass tank.

Collection of Duckweed and Soybean

Fresh duckweed was harvested from Lake View Park, Islamabad with the help of hand net and transported in nylon bags to laboratory. The duckweed were washed, dried and then stored in refrigerator (-20°C) in the form of paste and used whenever required for fish feed preparation. Dry soybean was collected from National Agricultural Research Council (NARC), Islamabad.

Feeding

Two different plant based diets containing soybean and duckweed as a major ingredient with a combination of rice polish, sunflower meal, gluten 30%, vitamin premix, Dicalcium phosphate, Carboxymethyl cellulose, fish meal, canola meal and wheat bran (Table 1). The 35% crude protein practical feeds were formulated following Pearson method [21]. For the preparation of 35% protein (based on protein requirement of selected fishes) practical diets all dry ingredients were powdered in a grinder and mixed with oil and water to make a paste. The paste was then passed through a meat grinder and pellets were obtained. The pellets were then oven dried, packed in plastic jars and stored in refrigerator. Feed was given on a daily basis at 4% of body weight.

Growth measurements

At the end of experiment, fish from each glass circular tank were captured, anesthetized with MS222 (60 mg/L) and weighed for determination of growth performance. Growth performances were assessed by using the following growth parameters:

i. Absolute Growth Rate (AGR) = Final weight – Initial Weight

ii. Relative Growth Rate% (RGR) = Final weight – Initial Weight/ Initial Weight × 100

iii. Specific Growth Rate% (SGR) = (ln final wet weight of fish - ln initial wet weight of fish) / Rearing period × 100

iv. Feed Conversion Ratio (FCR) = Total dry feed consumed (g) / Total wet weight gain (g)

v. Feed Conversion Efficiency% (FCE) = 1/ FCR × 100

vi. Hepato Somatic Index% (HSI) = (Liver weight (g) / Fish weight (g))× 100

Growth Hormone Assay (GH)

The Blood was taken from the caudal puncture and centrifuged at 10,000 rpm for 15 min and was stored at -20 °C for further Growth Hormone assays. Growth hormone concentrations of fishes fed F_{SBM} and F_{DW} diet were assessed with the help of Amgenix Micro LISA^{TM}-HGH Kit, USA. First, each well of ELISA plate was marked for sample recognition. Blood serum samples were thaw at room temperature and then centrifuge at 4˚C for 10 min. After that, required numbers of wells were secured in holder and 50 µl of every sample were added into wells and then 100 µl of enzyme conjugate reagent was added to every well. The wells were mixed properly for 30 seconds and then incubated at room temperature for 60 min. After that, the incubation mixture was removed through flicking plate in a waste container and rinsed. The microtiter wells were flicked five times with washing buffer. Then wells were struck strongly on absorbent paper for the removal of the residual water drops. After that TMB substrate (100 µl) was added to each well than mixed for 5 sec and incubated at room temperature for 20 min in dark. Stop salutation (100 µl) was added to stop the reaction. Then mixed for 30 sec, ensure that the blue color changes into yellow. Optical densities were read at 450 nm with a microtiter reader in 30 min.

Data Analysis

Data obtained from the feeding trial was expressed as mean ± S.E. The results were analyzed using ANOVA and MANOVA followed by Post Hoc Tukey Test. Values of $P < 0.05$ were considered statistically significant. All statistical calculations were performed using SPSS ver 23.0.

Results

Growth Performance

The results of growth performance and% survival rate of Grass carp and Silver carp fed with soybean and duckweed in

90 days of feeding trial are shown in Table 2 and Fig 1. During the experiment, no mortality of fish was observed. The survival rate of both fishes remained unchanged after feeding F_{SBM} and F_{DW} diets. The final weight of fingerlings of Grass carp fed F_{DW} was significantly higher ($P<0.001$) 22.08 ± 0.033 g as compared to Grass carp fed F_{SBM} 17.86 ± 0.033 g in monoculture system while the same results were also found in polyculture. The final body weight of fingerlings Grass carp fed on duckweed were 15.84 ± 0.023 g, considerably higher ($P<0.001$) as compared to fish fed on soybean (12.84 ± 0.013 g). When comparison was made between fingerlings of Grass carp in monoculture and polyculture, it was observed that% WG of the fingerlings of Grass carp fed F_{DW} in a monoculture was significantly higher (305.66 ± 0.013%) than Grass carp in polyculture (180.05 ± 0.023%).

Conversely, the final weight of the fingerlings of Silver carp fed on F_{SBM} was significantly higher ($P<0.001$) 10.35 ± 0.010 g, as compared to Silver carp fed F_{DW} 9.74 ± 0.023 g in monoculture system, while the same results were also found in polyculture. The final body weight of Silver carp (12.56 ± 0.013 g) was observed after fed F_{SBM} diet as compared to (10.23 ± 0.020 g) after fed F_{DW} diet (Table 2). The same trend was observed in% WG and% SGR (Table 2). Grass carp fed F_{DW} diets in monoculture and in polyculture showed significantly higher% SGR compared to Silver carp. The FCR value of Grass carp in both monoculture and polyculture was improved when fish offered F_{DW} diet compared to F_{SBM} diet. In case of Silver carp, F_{DW} diet showed the negative impact on FCR and% FCE. The% FCE of Silver carp cultured in monoculture and polyculture fed on F_{SBM} diet were considerably higher as compared to fish fed F_{DW} diets. In this study, no profound effect was observed in% HSI value in all experimental fish groups.

Growth Hormone

The growth hormone levels of the fingerlings of Grass carp fed F_{DW} were significantly ($P<0.05$) higher in both culture systems. In comparison to Grass carp, significantly higher level of GH was observed in fingerlings of Silver carp reared under both culture systems when fed F_{SBM} diet. Grass carp showed less growth hormone concentration after feeding F_{SBM} diet as compared to duckweed formulated feed whereas, Silver carp showed less concentration of growth hormone after feeding F_{DW} diet (Table 3; Fig 2).

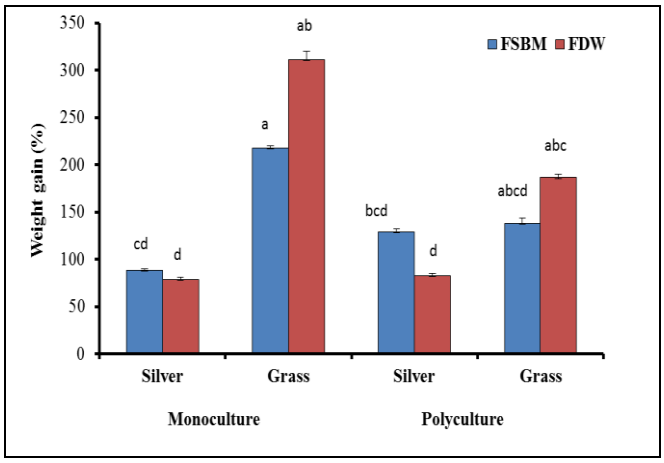

Fig 1: Weight gain (%) of fingerlings of Grass carp and Silver carp reared in monoculture and polyculture system on duckweed and soybean based diets. Data are represented as mean ± SE (n=21). Comparison was made between FSBM vs FDW (MANOVA followed by Tuckey Test)

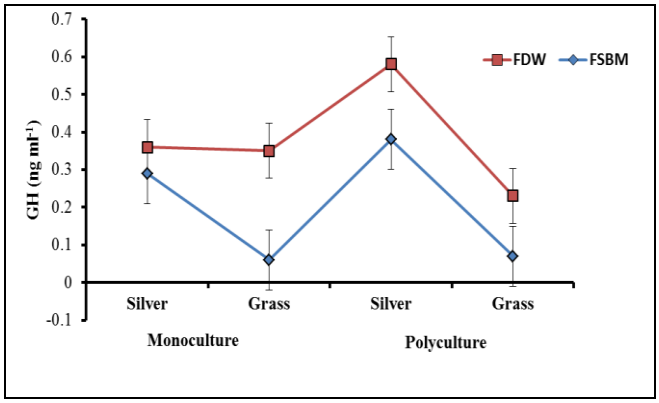

Fig 2: Growth Hormone concentration in fingerlings of Grass carp and Silver carp reared in monoculture and polyculture systems on duckweed and soybean based diet. Data are represented as mean ± SE (n=21). Comparison was made between F_{SBM} vs F_{DW} (ANOVA followed by Tuckey Test)

Table 1: Formulation of experimental diets

Ingredients	Percentage (%)
Fish meal	10.6
Soybean meal/Duckweed	21.3
Sunflower meal	21.3
Canola seed meal	21.3
Rice polish	5.3
Wheat bran	5.3
Gluten	10.0
Carboxymethyl cellulose	1.0
Dicalcium phosphate	1.0
Vitamin premix[a]	2.0
Vegetable oil	0.8

[a](Vitamin premix contains vitamins, aminoacids and minerals premix kg^{-1})

Vitamin AB.P 40,000,000IU, Vitamin D3B.P 820,000IU, Vitamin EB.P 6200mg, Vitamin K3B.P 800mg,Vitamin B2B.P 2500mg, Vitamin B3B.P 5100mg, Vitamin B12B.P 1000mg, Vitamin PP B.P 10,500mg, L. lysine B.P 10,500mg, DL- Methionine B.P 50,500 mg, Choline chloride USP 125,500mg, Manganese USP 30,000mg, Iron 15,100mg, Zinc USP 17,555mg, Copper B.P 1000mg, Cobalt B.P 50mg, Iodine B.P 300mg, Selenium B.P 80mg

Table 2: Effects of experimental diets on growth performance of fingerlings of Silver and Grass carp reared in monoculture and polyculture. Mean values ± SE (n=21). Mean values having similar superscripts in each row are insignificantly different (P > 0.05) from each other

	Monoculture				Polyculture			
	Silver carp		Grass carp		Silver carp		Grass carp	
	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}	F_{DW}	F_{SBM}
IBW (g)	5.54 ± 0.006^{bc}	5.48 ± 0.006^{ab}	5.55 ± 0.006^{c}	5.44 ± 0.009^{a}	5.64 ± 0.017^{d}	5.73 ± 0.020^{e}	5.63 ± 0.014^{d}	5.65 ± 0.014^{d}
FBW (g)	10.35 ± 0.010^{c}	9.74 ± 0.023^{a}	17.86 ± 0.033^{g}	22.08 ± 0.033^{h}	12.56 ± 0.013^{d}	10.23 ± 0.020^{b}	12.84 ± 0.013^{e}	15.84 ± 0.023^{f}
WG (%)	86.63 ± 0.030^{c}	77.45 ± 0.016^{a}	222.15 ± 0.020^{g}	305.66 ± 0.013^{h}	123.26 ± 0.013^{d}	79.23 ± 0.030^{b}	127.27 ± 0.006^{e}	180.05 ± 0.023^{f}
SGR (% b.wt./d)	0.45 ± 0.020^{a}	0.35 ± 0.023^{a}	0.96 ± 0.020^{c}	1.23 ± 0.026^{d}	0.63 ± 0.026^{b}	0.36 ± 0.023^{a}	0.65 ± 0.020^{b}	0.85 ± 0.016^{c}
FCR	2.91 ± 0.088^{cd}	3.14 ± 0.171^{d}	1.85 ± 0.115^{a}	1.58 ± 0.202^{a}	2.06 ± 0.145^{b}	3.27 ± 0.202^{d}	2.75 ± 0.256^{bcd}	2.28 ± 0.131^{abc}
FCE (%)	32.87 ± 2.933^{a}	31.37 ± 2.153^{a}	50.18 ± 2.179^{bc}	60.10 ± 2.594^{c}	47.55 ± 3.543^{b}	31.65 ± 0.965^{a}	33.77 ± 1.065^{a}	42.29 ± 1.292^{ab}
HIS (%)	1.48 ± 0.468^{a}	1.62 ± 0.095^{a}	0.75 ± 0.261^{a}	0.77 ± 0.043^{a}	1.27 ± 0.333^{a}	1.51 ± 0.614^{a}	1.11 ± 0.363^{a}	1.01 ± 0.052^{a}

Table 3: Effect of practical diet on growth hormone levels in fingerlings of Silver carp and Grass carp reared in monoculture and polyculture

	Monoculture Polyculture			
	Silver carp	Grass Carp	Silver carp	Grass Carp
F_{SBM}	0.291 ± 0.039^{bc}	0.057 ± 0.032^{a}	0.384 ± 0.025^{c}	0.065± 0.019^{a}
F_{DW}	0.069 ± 0.038^{a}	0.289 ± 0.076^{bc}	0.200 ± 0.038^{abc}	0.160 ± 0.020^{ab}

Mean values ± SE (n=21)

Discussion

The results from this study are very encouraging to use duckweed in a very safe and economical manner in fish feed. In the present study, no mortality of the fish was observed which shows that culture conditions were favorable for the growth and survival of fingerlings of the Silver carp and Grass carp. After 90 days of feeding trial, significantly higher (P<0.001) final weight (g),% WG,% SGR and% FCE of the Grass carp was observed after fed F_{DW} and reared in both monoculture and polyculture as compared to Grass carp fed F_{SBM} diet.

Duckweed (*Lemna s*p.) is considered as highly nutritious vegetative food for Grass carp because of its high-protein content and softness [24]. It is reported that 225-589 g of Grass carp can assimilate an average of 65-67% consumed duckweed, including 61% of available energy and 80% of crude protein [25]. In another study, Grass carp showed a significantly higher growth rate when fed duckweed than chara (*Chara* spp.) and southern naiad (*Najas guadalupensis*) [16]. In a study, faster growth rate of Grass carp i.e. 1.15 g per day when fed duckweed than other diets was also observed [26]. Similarly, 380 g Grass carp showed a significantly higher growth rate (6.1 g/d) when reared on duckweed as compared to 1.9 g/d) when fed pelleted feed [27].

The low growth rate of Grass carp when fed with soybean in this study may be due to the fact that soybean meal contains antinutritional factors like phytic acid, trypsin inhibitor, antigenic factors, oligosaccharides (stacchyose, raffinose), saponins, lectins, lipoxidase, goitrogens and phytoestrogens. Moreover, the final weight (g),% SGR,% WG and FCE% of fingerlings of Silver carp fed F_{SBM} was significantly higher (P<0.001) in both monoculture and polyculture. A significantly lower (P<0.001) FCR was found in Silver carp fed F_{DW} diet. The high growth rate of Silver carp as compared to Grass carp when fed soybean instead of duckweed may be due to the fact that Silver carp is a filter feeder and omnivore in nature and can't digest aquatic plants as efficiently as Grass carp. The Grass carp is a herbivore fish and digest plant based feed very easily as compared to Silver carp hence it shows high growth rate when fed duckweed as compared to Silver carp. Silver carp can only consume algae/plants in feed by mechanical crushing with the help of its pharyngeal teeth for breaking plant cell wall.

In the present study, it was observed that Silver carp in polyculture showed a higher growth rate as compared to monoculture system. It may be due to the fact that Silver carp can efficiently utilize defecated or waste matter of Grass carp. In polyculture, Grass carp consume low value vegetative wastes and as a result produce natural feed in the pond by fecal production and nutrient recycling [28].

In this study, significantly (P<0.05) higher growth rates were recorded for fingerlings of Grass carp (0.04 ± 0.03) in monoculture and (0.02 ± 0.02) in polyculture fed F_{DW} compared to the growth rates attained by fingerlings of Silver carp (0.19 ± 0.04) in monoculture system and (0.31 ± 0.03) in polyculture fed F_{SBM}, thus in combination Silver carp showed more GH concentration as compared to monoculture. Results reported in the present study are in agreement with the Pandit *et al*. [27] who revealed that Nile tilapia (omnivore) feeds directly on the waste material of Grass carp (herbivore) in polyculture and showed better growth performance as compared with monoculture system. It was also found that Grass carp showed higher growth after fed F_{DW} followed by Silver carp in polyculture this may be due to increasing concentration of growth hormone in both fish species. However, there is no previous study on effect of GH concentrations on these selected fish species, thus the present study is the first report on this aspect.

Conclusion

It may be concluded from the present study, that the better growth performance and GH concentration of F_{DW} diet are the ipso facto reveal that the duckweed can be used in fish feed formulation for cost-effective and better fish growth of the selected species. But, further studies are warranted to study the use of duckweed in fish feed in other important food

fishes.

Acknowledgments
The authors are thankful to Head, Department of Animal Sciences, Quaid-i-Azam University, Islamabad, Pakistan for providing necessary facilities for the study. The authors are also grateful to the Attock and Rawal fish hatchery for giving free of cost fish seed for the present study.

References

1. FAO (Food and Agriculture Organization). The State of World Fisheries and Aquaculture. Food and Agriculture organization, Rome, Italy, 2014.
2. Luo Z, Liu YJ, Mai KS, Tian L, Liu D, Tian XY. Optimal dietary protein requirement of grouper *Epinephelus coioides* juveniles fed isoenergetic diets in floating net cages. Aquac Nutr. 2004; 10:247-252.
3. Deng F, Koshio S, Yokoyama S, Bai SC, Shao Q, Cui Y *et al*. Effects of feeding rate on growth performance of white sturgeon (*Acipenser transmontanus*) larvae. Aquaculture. 2006; 217:589-598.
4. Halver JE. Fish Nutrition. In: J. E. Halver (ed). Academic Press, London, 1972.
5. Steffens W. Principles of fish nutrition. New York: Halstead press, 1989.
6. Pillay TVR. Aquaculture - Principles and Practices (Fishing News Books) 1st Edition, 1993, 600.
7. Bairagi A, Ghosh KS, Sen SK, Ray AK. Duckweed (*Lemna polyrhiza*) leaf meal as a source of feedstuff in formulated diets for rohu (*Labeo rohita*) fingerlings after fermentation with a fish intestinal bacterium. Bioresour Technol. 2002; 85:17-24.
8. Ahamad MU, Swapon MRS, Yeasmin TU, Raham MS, Ali MS. Replacement of sesame oil cake by Duckweed (*Lemna minor*) in broiler diet. Biol Sci. 2003; 6(16):1450-1453.
9. Uddin MN, Rahman MS, Shahjahan M. Effects of duckweed (*Lemna minor*) as supplementary feed on monoculture of GIFT strain of tilapia (*Oreochromis niloticus*). Progress Agric. 2007; 18:183-188.
10. Chowdhury MMR, Shahjahan M, Rahman MS, Sadiq-ul–Islam M. Duckweed (*Lemna minor*) as supplementary feed in monoculture of Nile tilapia (*Oreochromis niloticus*). J Fish Aquat Sci. 2008; 3:54-59.
11. Aslam S, Zuberi A, Kalhoro MA, Sarwar H, Shoaib A. A comparative study on growth performace of Chinese carps by using soybean, *Glycine max* (L) and duckweed, *Lemna minor* (L) meals as protein source. Sci. Int. 2016; 28(1):299-306.
12. Porath D, Helphor B, Koton A. Duckweed as an aquatic crop: Evaluation of clones for aquaculture. Aquat Bot. 1979; 7:273-278.
13. Chaturvedi KMM, Langote MDS, Asolekar RS. Duckweed- fed fisheries for treatment of low strength community waste water. WWW TM Newsletter- Asian Institute of Technology, India, 2003.
14. Bornali K. Effect of duckweed (*Lemna minor*) as supplementary feed on monoculture of tilapia (*Oreochromis niloticus*). MS thesis. Mymensingh: Bangladesh Agricultural University, 2004, 104.
15. Haque MS. Use of duckweed (*Lemna minor*) as supplementary feed in monoculture of sharpunti (*Puntius gonionotus*). MS thesis. Mymensingh: Bangladesh Agricultural University. 2005, 94.
16. Tao X, Yang F, Yao X, Yan-ling J, Xin-rong M, Yun Z *et al*. Comparative transcriptome analysis to investigate the high starch accumulation of duckweed (*Landoltia punctata*) under nutrient starvation. Biotechnol Biofuels. 2013; 6:1-15.
17. Leng RA, Stambolie JH, Bell R. Duckweed a potential high protein feed resource for domestic animals and fish. Livestock Research for Rural Development. 1995; 7:1.
18. Goetz H. The Effects of Baking on the Action of Trypsin inhibitor. M.Sc thesis. The Ohio State University 2012, 1-12.
19. Hertampf JW, Piedad-Pascual F. Handbook on Ingredients for Aquaculture Feeds. Kluwer Academic Publications, Dordrecht, 2000.
20. Aslam S, Abbas S, Kalhoro MA, Shoaib A. Anchor worms (lernaeid parasites) *Lernaea polymorpha* Yu and *Lernaea cyprinacea* L (Copepode: Lernaidae) on major carps at different fish farms in Punjab, Pakistan. Sci. Int. 2016; 28:295-298.
21. Reddy PVGK, Gjerde B, Tripathi SD, Jana RK, Mahapatra KD, Gupta SD *et al*. Growth and survival of six stocks of rohu (*Labeo rohita*) in mono and polyculture production systems. Aquaculture. 2002; 203:239-250.
22. Reineke M, Bjornsson BT, Dickoff WW, McCormick SD, Navarro I, Power DM. Growth hormone and insulin-like growth factors in fish: where we are and where to go. Gen Comp Endocrinol. 2005; 142:20-24.
23. Rezaei A. Vaiation in growth hormone (GH) of gene in exon sequence in three salmon types. Biol Sci. 2012; 4(1):43-53.
24. Kent D. M.Sc thesis on Potential effects of two Asian Carp species on Pallid Sturgeon. Linclon, 2012, 1-16,
25. Yilmaz E, Ekyurt I, Gunal G. Use of Duckweed, *Lemna minor*, as a Protein Feedstuff in Practical Diets for Common Carp (*Cyprinus carpio*) Fry. J Fish Aquat Sci. 2014; 4:105-109.
26. Sutton DL. Grass carp (*Ctenopharyngodon idella*) in North America. Aquat Bot. 1977; 3:157-164.
27. Pandit NP, Shrestha KM, Yi Y, Diana SJ. A report on polyculture of grass carp and Nile Tilapia with Naiper grass as the sole nutrient input in the subtropical climate of Nepal, 2012, 16.
28. Pipalova I. A Review of Grass Carp Use for Aquatic Weed Control and its Impact on Water Bodies. J Aquat Plant Manag. 2006; 44:1-12.