A COMPARATIVE STUDY ON GROWTH PERFORMANCE OF CHINESE CARPS BY USING SOYBEAN, *GLYCINE MAX* (L) AND DUCKWEED, *LEMNA MINOR* (L) MEALS AS PROTEIN SOURCE

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***ABSTRACT** : Development of ecofriendly and efficient fish feed for best growth performance is an important step in the field of fisheries and aquaculture. A feeding trial was conducted to compare the effect of Duckweed (Lemna minor) and soybean Glycine max (L) meals as a source of protein on the growth performance, growth hormone level and proximate composition of Grass carp, Ctenopharyngodon idella (Valenciennes, 1844) and Silver carp, Hypophthylmichthys molitrix (Valenciennes, 1844) in monoculture system and in combination. Two 35 % protein experimental diets, F_{SBM} (feed containing 21 % soybean meal) and F_{DW} (feed having 21 % duckweed) as a source of protein were prepared and fed for 12 weeks. At the end of experiment, juveniles of C. idella showed a non-significantly higher growth rate as compare to H. molitrix when fed F_{DW} while H. molitrix 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 growth hormone level of Juvenile C. idella fed F_{DW} were significantly ($P<0.05$) higher in both culture systems while same trend was shown by H. molitrix when fed F_{SBM} diet. Proximate analysis showed approximately similar composition of both diets. The results of this study indicate the use of duckweed as a protein source in fish feed.*

Keywords: Chinese carps; Combination; *Glycine max; Lemna minor;* Monoculture; Proximate analysis

INTRODUCTION

Duckweed is a common name of an aquatic plant family Lemnacea. It includes 5 genera namely: *Lemna, Wolffia, Spirodella, Wolffiella and Landoltia* with >40 species. It is amongst the smallest of the flowering plants in the world and is widely distributed in tropical and temperate regions of the world. It is found floating on the surface of water bodies. Duckweed has been used to feed different animals, including fish [3]. It has been accepted in fish feed as protein rich diet (40-45% dry weight) [33]. The grass carp weight could be tripled (100 to 300 g) within only 50 days when feeding a mash of *L. minor* and *L. gibba* [24]. It is better to store soybeans for human use and to search other plant protein sources for higher production yield [20]. There is a diversity in plant protein rich sources which could replace soybean meal in animal feed in Asia among these duckweed is a protein rich source can be produced cheaply from waste water [7]. The duckweed contains high protein content, about 400 g/kg, closely resembles soybean meal and also has well balanced amino acid profile, particularly lysine (6.9 g/100 gm protein) which is a limiting amino acid in other plant proteins [33]. Many investigators reported that many herbivorous fishes consume duckweed readily because the cell wall of this plant lack lignin, therefore, enhance digestibility and considered as an ideal source of feed [30]. Many researchers considered duckweed as a super ingredient for utilization in feed for many reasons like: high protein content, high mineral absorption capability, easy harvest ability , low lignin & fiber content, extending harvesting & growing periods, susceptibility to few pests and non-toxic to domestic stock. It floats on the surface of water so it can be easily harvested with the help of skimming device. Duckweed experiences only a short period of dormancy as it often grows year round. It shows an apparent immunity for many pests associated with other forages that's why it will reduce the cost for pest management which is necessary in other agricultural crops. It contains 92-95% water [15].

Duckweed has the ability to double its growth rate in 16 hrs to 4 days under suitable conditions. The high protein content (41% crude protein) in duckweed is similar to that of soybean meal and it also contains pigments and carotenoids which can promote crustacean growth [12]. The amino acid profile of *Spirodela* and *Lemna* can be comparable favorably with that of peanut meal and soybean meal. The duckweed (*L. minor*) if grown under suitable conditions and harvested regularly can have 35% protein content and 5-15% fiber content depending on the species [33]. The protein content has a higher concentration of methionine, lysine and the EAA as compared to most plant protein sources. Its leaves contain very low amount of fiber, therefore even monogastric animals can digest it [4].

Soybean meal *(Glycine max)* is one of those interesting alternatives used for fish growth as it contains high protein content and adequate amino acid profile. However, it has many anti-nutritional factors: trypsin inhibitor, phytic acid, oligosaccharides (raffinose, stachyose), antigenic factors, lectins, saponins, lipoxidase, phytoestrogens, and goitrogens which limit its use for fish [10]. The reduced growth with *G. max* might be due to its sub optimal amino acid balance [9] and also due to the presence of anti-nutritional factor tannin especially trypsin inhibitor in soybean meal [11]. Very low amount of tannin as 0.5% in the diet can cause reduced growth in poultry and the toxicity of tannin to fish has also been reported [5].

Different carps having different feeding habits are reared in combination in the same water bodies. This system shows higher production rate and higher economic and financial viability because natural productivity can be fully utilized hence the monoculture system is less frequently practiced, but in many countries, monoculture systems are also practiced because certain consumer preferred fish species

which shows higher production and survival rate when reared independently [25]. It is being practiced throughout the world in different environments using different fish species ranging from mono to polyculture systems [33].

An increase in growth rate shows the higher secretion of growth hormone that is a polypeptide hormone which is secreted from pituitary gland. It is required for development and normal growth. Growth hormone together with chorionic somatomammotropin, form a family of peptide hormones of identical function and structure [21]. In aquaculture, growth rate of fish is very important factor and it is involved in food conversion; linear growth and appetite in fish enhance somatic growth in vertebrates including teleost [26].

Feeding habits directly involved in the chemical composition of the fish's body. The material is subjected to a series of simple chemical tests, as to determine the content of moisture, ash, crude protein, crude fiber, lipid and digestible carbohydrates [19]. For routine analysis of fish, proximate composition is a good indicator of physiology [27]. Age and body size has also been shown to have a main effect on body composition. Many studies related the changes in the composition of different organs or muscle tissues to feeding frequency, age, migration, sex, starvation, season and temperature [1].

The study was design for testing the hypothesis **"**duckweed grown as a component of an integrated farming system will be a more economical supplement than soybean meal". The objectives of the study were threefold. Firstly, to evaluate the growth performance of juvenile silver carp, *Hypophthalmichthys molitrix* (Valenciennes, 1844) and grass carp, *Ctenopharyngodon idella* (Valenciennes, 1844); secondly, to determine growth hormone level of these Chinese carps and lastly to evaluate the chemical composition of feed and flesh of these carps under monoculture as well as a combination by replacing *Glycine max* (L) with *L. minor* (L) in fish feed as a cheap source of crude protein.

MATERIALS & METHODS:

The experiment was conducted for a period of 90 days in glass aquaria (60× 30 ×30 cm) for monoculture and (90 × 45 × 45 cm) for combination. Juvenile *H. molitrix* and *C. idella* (mean ± S.E, body mass 9.84 ± 0.08 g and length 11.27 ± 0.03 cm respectively) were used and equally distributed in 9 glass aquaria, 3 for *H. molitrix* and 3 for *C. idella* while remaining 3 were for combination purpose. Experimental work was conducted in triplicate and fishes were stocked at a stocking density of 2.5 g L^{-1} at the ratio of 15:15. All aquaria were fitted with heaters and air stones for optimum temperature (26°C) and DO level.

Area of Study:

The experimental work was executed at Fisheries and Aquaculture Lab. in Quaid-i-Azam University (QAU), Islamabad, Pakistan.

Collection of Duckweed

L. minor was collected from Lake View Park, Islamabad with the help of hand net and transported in nylon bags to Fisheries and Aquaculture Lab, QAU, Islamabad. For the removal of pathogens, *L. minor* was oven dried for only 10 min at 100 °C. This drying procedure is very effective because it removes large amount of water and pathogens, not destroy plant cells and not burn the sample while crude protein is also not affected [17]. After that *L. minor* stored in refrigerator (-20 °C) in the form of paste and used whenever required for fish feed preparation.

Feeding

Two different plant based diets including *Glycine max* and *L. minor* as major content with a combination of rice polish, sunflower meal, gluten 30%, vitamin premix, Dicalcium Phosphate, Carboxymethyl cellulose, fish meal, canola meal, wheat bran were given to the fishes. The 35% crude protein experimental feeds were formulated by following Pearson method [25]. For the preparation 35% protein experimental diets all dry ingredients were ground in a grinder and mixed with oil and water to make a paste which was then passed through a meat grinder and pellets were formed. 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.

Proximate Analysis:

The complete methods for analysis of feed and body compositions are given as follow:

Dry matter and Moisture Content:

A china dish was taken, washed and placed in oven at 105 °C for ten minutes. Then china dish was placed in desiccator for cooling. Then empty china dish was weighed on digital balance. Then 5 g/10 g sample was weighed again in that china dish (B) and was placed in oven at 105 °C for 24 hours. After that, china dish was placed in desiccator and again weighted (C).

The dry matter was estimated as follow:

Dry Matter (%) = Weight of sample after drying (C)/ Weight of sample before drying (B) × 100

Moisture (%) = 100 – Dry matter (%)

Crude Ash:

A clean crucible was placed in a muffle furnace oven for 1 hour at 100 °C. Then crucible was transferred to desiccator and cooled down at room temperature. Then empty crucible dish was weighed and 2-4 g sample (oven dried) was placed into a pre-weight crucible dish. Then crucible was again placed in a muffle furnace for 24 hours at 550-600 °C. After that transferred into desiccator and cooled to room temperature. Crucible contain ash was weighed as quickly as possible to prevent moisture absorption. Then ash was saved for mineral analysis.

The crude ash was estimated as follows:

Crude Ash (%) = weight of ash / weight of sample × 100

Crude Protein:

The evaluation of the samples was done by micro Kjeldahl's method. 1-2 g of sample (oven dried) was mixed with 5 g Digestion Mixture (Mixing 1 part of Na_2SO_4 (01 g) in 10 parts of $CuSO_4$ (10 g). Then 30 ml of conc.H_2SO_4 was added to digest the sample. Heated for 2-3 hours on hot plate at 250 °C in a fume hood until white to light green color appeared. Then it was cooled down and distilled water was added to make a final volume of 250 ml in a volumetric flask. Then 10 ml of distilled sample was mixed with 10 ml of 40% NaOH solution in Kjeldahl apparatus. Funnel plugged firmly and heated for 3 min, then added 10 ml (2%) boric acid solution Liberated distillate (Ammonia) was collected in 10 ml Boric acid (2%) of pink color contain a drop of indicator

(methyl red). After the appearance of golden yellow color, titration was done by using H_2SO_4 (0.1N).

Percentage of Nitrogen calculated as:

Percentage of Nitrogen = Normality of H_2SO_4 × Volume of H_2SO_4 used × 250 x 0.014 × 100 / Weight of sample × 10

Whereby;

250 = Dilution of the digested mixture

0.014 = Standard Volume of H_2SO_4 (0.1N) to neutralize 1ml ammonia

10 = used volume of the diluted mixture

100 = for percentage of Nitrogen

By following formula Crude protein in the sample was estimated.

Percentage of Crude Protein = Percentage of Nitrogen × 6.25

Where,

6.25 = Assumed factor to calculate Crude Protein from N (%).

Total Fats:

To determine the total fat contents of sample, Hexane extraction method was used through Soxthlet apparatus. About 3 g of moisture free sample (oven dried) was put in the Soxthlet apparatus thimble. Then thimble was positioned in an extractor and placed correctly under the condenser of extraction apparatus. 150 ml solvent was poured into the receiving flask and connected it to the apparatus. Tuned on the heater and continued the supply of water. Extraction was done for 10 hours at the rate of 3-4 drops/sec. After that thimble was removed from the extractor and was allowed to evaporate and dry. The thimble was weighed after extraction.

Percentage of total fat in the sample was determined as:

Total fats (%) = Wt. of thimble after evaporation – Wt. of empty thimble / Wt. of sample × 100

Crude Fiber:

For the determination of crude fiber, the organic residue which was left after the sequential extraction of feed with ether was used. It was transferred to the flask and 200 ml of pre-heated H_2SO_4 (1.25%) was added and gently boiled for 30 min. For maintaining the constant volume of acid, hot water was added. The Whatman filter paper was fitted with funnel by the addition of hot water into the funnel. The boiled acid sample was filtered into the funnel. The residue was washed several times with hot water until the neutral color of litmus paper appeared, then transferred back to the beaker. Then 200 ml of pre-heated Na_2SO_4 (1.25%) was added and boiled for only 30 min. Then residue was filtered and dried at 65 °C for just 2 hrs and weighed. Then residue was transferred to crucible and placed in a muffle furnace for 4 hrs at 400-600 °C, then cooled in a desiccator and weighed.

The % of total crude fiber was determined as follows:

Crude fiber (%) = Dry wt. of residue before ash – Wt. of residue after ash/ wt. of sample × 100

Nitrogen Free Extract (NFE):

The amount of total carbohydrates was found out as follows:

NFE (%) = 100 – (Crude Protein + Crude fiber + Total Ash + Total Fats)

Sampling and Growth measurements

Growth performances were assessed by using the following growth parameters.

Weight gain (AGR) = $W_f - W_i$

RGR (%) = $W_f - W_i / W_i \times 100$

SGR (%) = (ln final wet weight of fish - ln initial wet weight of fish) / Rearing period × 100

FCR = Total dry feed consumed (g) / Total wet weight gain (g)

FCE (%) = 1/ FCR × 100

HSI (%) = (weight of the liver of fish / Whole body weight of fish × 100)

At the end of experiment, fish from every aquarium were captured, anesthetized with MS222 (60 mgL^{-1}) and weighed for determination of growth performance. Blood was withdrawn from the caudal puncture and centrifuged at 10,000 rpm for 15 min and was stored at −20 °C for further GH assays.

Growth Hormone Assay (GH)

Growth hormone concentrations of fishes fed F_{SBM} and F_{DW} diet were assessed with the help of Amgenix MicroLISA™-HGH Kit, USA. First, each well of ELISA plate was marked for samples 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 taken 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. Then TMB substrate (100 µl) was added to each well than mixed for 5 sec and incubated at room temperature for 20 min in dark. After that, stop salutation (100 µl) was added to stop the reaction. Then mixed for 30 sec, ensure all the blue color changed into yellow. Optical densities were read at 450 nm with a microtiter reader in 30 min.

Data Analysis

Data obtained from the experiment was expressed as mean ± S.E. The results were analyzed using one-way ANOVA followed by LSD Test. Values of $P<0.05$ were considered statistically significant.

RESULTS:

Proximate Analysis

The chemical composition of both Experimental diets F_{SBM} and F_{DW} was approximately similar (Table 1).

The moisture content of *H. molitrix* fed F_{SBM} diet was significantly ($P<0.05$) higher 77.86 ± 0.34 % than fish fed F_{DW} diet, 75.02 ± 0.37 % while moisture content of *C. idella* fed F_{DW} was significantly ($P<0.05$) higher 77.14 ± 0.42 % as compared to fish fed F_{SBM} 70.72 ± 0.49 %. The dry matter content of *H. molitrix* fed F_{DW} diet was also significantly ($P<0.05$) higher 25.57 ± 0.33 % as compared to fish fed F_{SBM} diet 22.83 ± 0.33 % while *C. idella* fed F_{SBM} had significantly ($P<0.05$) higher dry matter 29.73 ± 0.33 % as compared to fish fed F_{DW} 23.42 ± 0.33 %. The ash content of *H. molitrix* and *C. idella* showed no significant difference in both F_{SBM} and F_{DW} feed 1.29 ± 0.35 %, 1.13 ± 0.36 % and 1.32 ± 0.33 %, 1.29 ± 0.30 %, respectively (Table 2).

The proximate analysis of duckweed revealed that it contained 94.68 ± 0.30 %, moisture, .83 ± 0.08 %, dry matter and 524.56 ± 0.45 % ash content (Table 3).

Growth Performance

The results of growth performance and % survival rate of *C. idella* and *H. molitrix* fed with *Glycine max* and duckweed in 90 days of feeding trial are shown in Table 4 and Fig 1. During the experiment, no mortality of fish was observed. The survival rate of both Chinese carps remained unchanged after feeding *Glycine max* (F_{SBM}) and *L. minor* (F_{DW}) and all the fishes survived. The final weight of juvenile *C. idella* fed F_{DW} was significantly higher ($P<0.001$) 39.45 ± 0.30 g as compared to *C. idella* fed F_{SBM} 32.03 ± 0.56 g in monoculture system while the same thing happened in combination. After 90 days of feeding trial, the final body weight of juvenile *C. idella* fed on *L. minor* were 41.39 ± 0.26 g, considerably higher ($P<0.001$) as compared to fish fed on *Glycine max*. When comparison was made between juvenile *C. idella* in mono and combination, it was observed that % WG of juvenile *C. idella* fed F_{DW} into a combination was 322.53 ± 5.54% significantly higher than *C. idella* in a monoculture system.

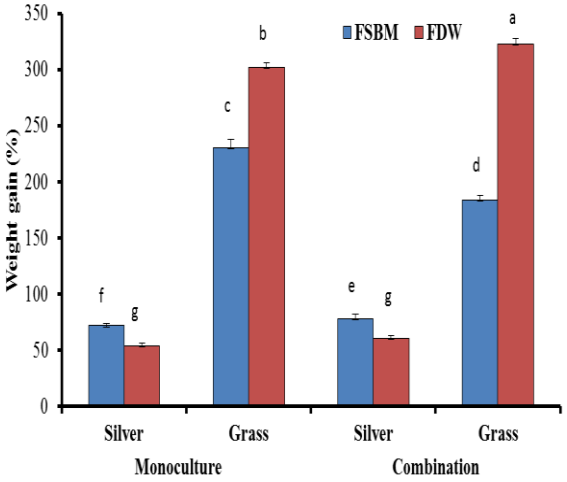

Fig 1.Weight gain (%) of Juveniles of Chinese carps reared in monoculture and combination on duckweed and soybean based diet. Data are represented as mean ± SE (n=21). Comparison made: F_{SBM} vs F_{DW}. (One-way ANOVA followed by LSD Test). F_{SBM}, Basal diet having 21% Soybean Meal; F_{DW}, Basal diet contains 21% Duckweed

Conversely, the final weight of juvenile *H. molitrix* fed on F_{SBM} was significantly higher ($P<0.001$) 16.38 ± 0.29 g, as compared to *H. molitrix* fed F_{DW} 14.51 ± 0.58 g in monoculture system, while the same thing happened in the combination where significantly higher ($P < 0.001$) final body weight of juvenile *H. molitrix* (18.04 ± 0.93 g) was observed after fed F_{SBM} diet as compared to 15.32 ± 0.58 g after fed F_{DW} diet (Table 4).

The same trend was observed in % WG and % SGR (Table 4). Juveniles' *C. idella* fed F_{DW} diets in monoculture and combination showed significantly higher % SGR compared to *H. molitrix*. The FCR value of *C. idella* in both mono and combination was improved when fish offered F_{DW} diet compared to F_{SBM} diet. In case of *H. molitrix*, F_{DW} diet showed the negative impact on FCR and % FCE. The % FCE of *H. molitrix* reared in mono and combination and fed on F_{SBM} diet were considerably higher as compared to fish fed F_{DW} diets.

No profound effect was observed in % HSI value in all experimental groups of fish.

Growth Hormone

The growth hormone level of juvenile *C. idella* fed F_{DW} were significantly ($P<0.05$) higher in both culture systems. In comparison to *C. idella*, significantly higher level of GH observed in Juvenile *H. molitrix* reared under mono and combination and fed F_{SBM} diet. *C. idella* showed less growth hormone concentration after feeding F_{SBM} diet as compared to duckweed formulated feed whereas *H. molitrix* showed less concentration of growth hormone after feeding F_{DW} diet (Table 5; Fig 2).

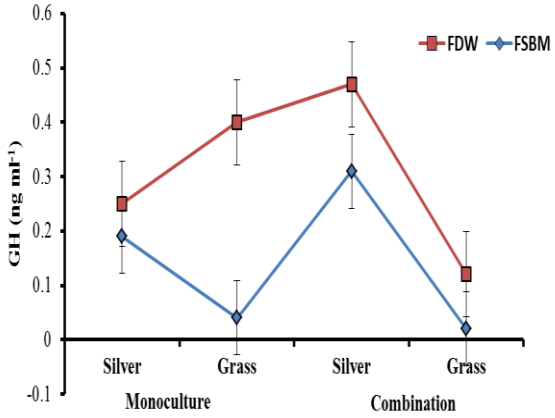

Fig 2.GH concentration in juvenile Chinese carps reared in monoculture and combination on duckweed and soybean based diet. Data are represented as mean ± SE (n=21). Comparison made: **F_{SBM} vs F_{DW}. (One-way ANOVA followed by LSD Test). F_{SBM}, Basal diet having 21% Soybean Meal; F_{DW}, Basal diet contains 21% Duckweed**

DISCUSSION:

The importance of knowledge about the chemical composition of feed used in fish nutrition technologies to enhance the growth performance of different fish species is a well-documented (Mahmud *et al*., 2012). In the present study, the proximate analysis of F_{SBM} and F_{DW} showed that these two fish diets had approximately the same chemical composition (dry matter, moisture content, ash, crude protein, lipid, crude fiber and nitrogen free extract). Therefore it appears that *L. minor* can replace *Glycine max* in fish feed in a very cost effective manner. The chemical composition of flesh of both fish species, i.e., *C. idella* and *H. molitrix* showed significant ($P<0.05$) difference in dry matter and moisture content, that may be due to the difference in feed utilization by these two Chinese carps but there was no significant difference in ash content. The results obtained in the present study of chemical composition of *L. minor* and fish flesh are in agreement with [33]. These results are very encouraging to use *L. minor* 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 or water quality parameters were favorable for the growth and survival of juvenile *H. molitrix* and *C. idella*. After 90 days of feeding

trial, significantly higher (P<0.001) final weight (g), % WG, SGR % , FCE% of the *C. idella* was observed after fed F_{DW} and reared in both combination and monoculture system as compared to *C. idella* fed F_{SBM} diet.

Duckweed (*Lemna sp*.) is considered as highly nutritious vegetative food for *C. idella* because of its high-protein content and softness [15]. It was observed that 225-589 g of *C. idella* can assimilate an average of 65-67%

Table 1: Chemical composition of Basal and Experimental Feed

Chemical composition	F_{SBM} (%)	F_{DW} (%)
Moisture	12.30	11.00
Dry Matter	87.70	89.00
Ash	10.50	11.50
Crude Protein	26.25	27.13
Crude Fiber	6.90	7.60
Lipid	18.00	20.50
Nitrogen Free Extract (NFE)	38.35	33.27

Table 2: Body composition of Chinese carps fed experimental diets fed diets with Duckweed and Soybean Meal for 90 days

	Silver Carp		Grass carp	
Components	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}
Moisture	$77.86 \pm 0.34^{***}$	$75.02 \pm 0.37^{***}$	$70.72 \pm 0.49^{*}$	$77.14 \pm 0.42^{*}$
Dry Matter	$22.83 \pm 0.33^{*}$	$25.57 \pm 0.33^{*}$	$29.73 \pm 0.33^{*}$	$23.42 \pm 0.33^{*}$
Ash	1.29 ± 0.35^{ns}	1.13 ± 0.36^{ns}	1.32 ± 0.33^{ns}	1.29 ± 0.30^{ns}

Data are represented as mean ± SE (n=21). *P<0.05, **P<0.01, ***P<0.001, ns= not significant. The comparison made between F_{SBM} vs $F_{DW.}$ (One-way ANOVA followed by LSD Test)

Table 3: Proximate composition of duckweed used in the experimental diets

Proximate composition	Duckweed
Moisture	94.68 ± 0.30
Dry Matter	5.83 ± 0.08
Ash	24.56 ± 0.45

$F_{SBM,}$ Basal diet having 21% Soybean Meal; F_{DW}, Basal diet contains 21% Duckweed

Table 4: Effect of experimental diets on growth performance of Juvenile Chinese carps reared in monoculture and Combination

	Monoculture				Combination			
	Silver carp		Grass carp		Silver carp		Grass carp	
	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}	F_{SBM}	F_{DW}
IBW(g)	9.73 ± 0.39^{d}	9.31 ± 0.29^{e}	9.85 ± 0.72^{c}	9.81 ± 0.29^{c}	10.31 ± 0.36^{a}	9.49 ± 0.44^{e}	10.00 ± 0.33^{b}	9.84 ± 0.45^{c}
FBW(g)	16.38 ± 0.29^{f}	14.51 ± 0.58^{h}	32.03 ± 0.56^{c}	39.45 ± 0.30^{b}	18.04 ± 0.93^{e}	15.32 ± 0.58^{g}	27.90 ± 0.69^{d}	41.39 ± 0.26^{a}
W.G (%)	72.03 ± 2.34^{f}	53.85 ± 2.41^{g}	230.26 ± 7.40^{c}	302.16 ± 3.59^{b}	78.15 ± 3.83^{e}	60.83 ± 2.72^{g}	183.71 ± 3.89^{d}	322.53 ± 5.54^{a}
SGR (% b.wt./d)	0.14 ± 0.01^{d}	0.03 ± 0.02^{e}	0.79 ± 0.02^{b}	0.98 ± 0.01^{a}	$0.17 \pm 0.02d^{e}$	0.07 ± 0.02^{de}	0.63 ± 0.01^{c}	1.03 ± 0.01^{a}
FCR	3.29 ± 0.07^{b}	3.81 ± 0.15^{b}	1.73 ± 0.03^{e}	1.50 ± 0.04^{f}	2.91 ± 0.12^{c}	3.63 ± 0.13^{a}	1.95 ± 0.02^{d}	1.53 ± 0.02^{f}
FCE (%)	30.45 ± 0.66^{e}	26.37 ± 1.09^{e}	57.95 ± 1.03^{d}	66.56 ± 1.71^{a}	34.47 ± 1.39^{d}	27.67 ± 0.99^{e}	51.24 ± 0.53^{c}	65.53 ± 1.04^{a}
HSI (%)	1.17 ± 0.50^{ab}	1.50 ± 0.59^{a}	0.39 ± 0.30^{b}	0.72 ± 0.04^{ab}	1.02 ± 0.44^{ab}	0.91 ± 0.44^{ab}	0.82 ± 0.03^{ab}	0.41 ± 0.04^{ab}

Data are represented as mean ± SE (n=21), the comparison made between F_{SBM} vs $F_{DW.}$ (One-way ANOVA followed by LSD Test), $F_{SBM,}$ Basal diet having 21% Soybean Meal; F_{DW}, Basal diet contains 21% Duckweed

Table 5: Effect of experimental diets on growth hormone level in Juveniles of Chinese carps reared in monoculture and combination

	Monoculture		Combination	
	Juveniles		Juveniles	
	Silver carp	Grass Carp	Silver carp	Grass Carp
F_{SBM}	0.19 ± 0.04^{bc}	0.04 ± 0.03^{d}	0.31 ± 0.03^{ab}	0.02 ± 0.02^{d}
F_{DW}	0.06 ± 0.03^{cd}	0.36 ± 0.11^{a}	0.16 ± 0.04^{cd}	0.10 ± 0.02^{cd}

Data are represented as mean ± SE (n=21); the comparison made between F_{SBM} vs $F_{DW.}$ (One-way ANOVA followed by LSD Test), $F_{SBM,}$ Basal diet having 21% Soybean Meal; F_{DW}, Basal diet contains 21% Duckweed

consumed *L. minor*, including 61% of available energy and 80% of crude protein [32]. In another study, *C. idella* showed a significantly higher growth rate when fed *L. minor* than chara (*Chara spp*.) and southern naiad (*Najas guadalupensis*) [30]. Sutton [29] also observed faster growth rate of *C. idella* i.e., 1.15 g per day when fed *L. minor* than other diets. Similarly 380-g *C. idella* showed a significantly higher growth rate (6.1 g d^{-1}) when reared on *L. minor* as compared to 1.9 g d^{-1}) when fed pelleted feed [22].

The low growth rate of *C. idella* when fed with *Glycine max* in our 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 as described by Iwashita *et al.,* [14]. Moreover, the final weight (g), % SGR, % WG and FCE % of juvenile of *H. molitrix* fed F_{SBM} was significantly higher ($P<0.001$) in both monoculture and combination and also significantly lower ($P<0.001$) FCR as compared to *H. molitrix* fed F_{DW} diet. The high growth rate of *H. molitrix* as compared to *C. idella* in the case of feeding *Glycine max* instead of *L. minor* may be due to the fact that *H. molitrix* is a filter feeder and omnivore in nature and can't digest aquatic plants as efficiently as grass carp. *C. idella* is a herbivore fish and digest plant based feed very easily as compared to *H. molitrix* hence it shows high growth rate with duckweed as compared to *H. molitrix*, filter-feeder. *H. molitrix* can only consume algae/plant in feed by mechanical crushing with the help of its pharyngeal teeth for breaking plant cell wall [8].

In the present study, it was observed that *H. molitrix* in combination showed a higher growth rate as compared to when grown in a monoculture system. It may be due to the fact that *H. molitrix* can efficiently utilize defecated or waste matter of *C. idella* whereas *C. idella* is a voracious fish species. In combination, *C. idella* consumes low value vegetative wastes and as a result produce natural feed in the pond by fecal production and nutrient recycling (Pipalova, 2006). The effectiveness reported in Chinese "one *C. idella* raises three *H. molitrix*" and a stocking ratio of 5:1 by weight is most preferable for *C. idella* and filter-feeding species in combination including *H. molitrix*, common carp (*Cyprinus carpio*) and bighead carp (*Aristichthys nobilis*) [2].

In the present study, significantly ($P<0.05$) higher growth rates were recorded for juvenile of *C. idella* 0.04 ± 0.03 in monoculture and 0.02 ± 0.02 in combination fed F_{DW} compared to the growth rates attained by juvenile of *H. molitrix* 0.19 ± 0.04 in monoculture system and 0.31 ± 0.03 in combination fed F_{SBM}, so in combination *H. molitrix* showed more GH concentration as compared to monoculture. *H. molitrix* lacks a true stomach hence it requires feed continuously [16] and it regularly feeds on *C. idella* feces in the combination. Results reported in present study are in agreement with the Pandit *et al*. [23], who revealed that Nile tilapia (omnivore) feeds directly on the waste material of *C. idella* (herbivore) in combination and showed better growth performance as compared with monoculture system. Present study revealed that *C. idella* showed higher growth after fed F_{DW} followed by *H. molitrix* in combination that result showed the definitely increasing concentration of growth hormone in both fish species. However there is no earlier study on GH concentration on these Chinese carps and the present study can be regarded as the first effort which was related to study of growth performance of these fish species and record the data on growth hormone.

Although, there is much need of further study to use *L. minor* in aquatic feed. But, if a farmer use *L. minor* in fish feed than using expensive source of protein, *G. max;* then he can gain more profit in a very good way by little input.

CONCLUSION:

In Conclusion, The better growth performance, high GH concentration, similar chemical composition to that of F_{SBM} diet are some of the evident facts which clearly indicates the use of *L. minor* in fish feed formulation for good fish performance.

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