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Effect of garlic (*Allium sativum*) supplementation on growth, survival and body composition of striped catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878)

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Abstract
Garlic contains crucial bioactive components and study on garlic concluded it as growth promoters, antioxidants, antimicrobial and improved digestibility and caraccas composition of fish. To evaluate the effect of garlic on growth, survival, feed utilization and proximate composition of *Pangasianodon hypophthalmus* this study was conducted using three doses (1, 2 and 3%) of garlic powder (GP) incorporated diets. *P. hypophthalmus* fry of initial size 85.20 ±0.84 mm and 5.47 ±0.30 g was reared in PVC plastic cages of size (2m x 1m x 1.2m) with density 240 numbers per cage using GP incorporated basal diets and basal diet without GP for 180 days. Significantly higher ($P<0.05$) final length, final weight, average daily gain (ADG), SGR, survival, yield, FCE, PER, APR, ALR and CP were observed in GP2% treated groups whereas the same group showed lower ($P<0.05$) FCR, VSI, HSI and body fat content. The present study revealed that dietary inclusion of GP2% can improve growth, feed utilization and flesh quality of *P. hypophthalmus*.

Keywords: *Pangasianodon hypophthalmus*, Pangasius fish, garlic powder, growth, survival, biochemical composition

Introduction

Pangasius is an important fresh water catfish known for its fast growth in aquaculture (Jeyakumari *et al.*, 2016) [26]. It is one of the commercially important fish belongs to the family Pangasidae (Pouyaud *et al.*, 2004) [46]. Naturally it is found in Chao Phraya River in Thailand and lower basin of Mekong River (Vidthayanon and Hogan, 2013) [64] mainly the Mekong basins in Cambodia, Lao People's Democratic Republic, Thailand and Vietnam, the Irrawaddy or Ayeyarwady Basin of Myanmar (Nguyen, 2013; FAO, 2020) [39, 17] also. It is famous with many common names *viz*. iridescent shark, catfish, striped catfish, sutchi, Pla Sawai in Thailand; Cá Tra, in Vietnam (Neilson *et al.*, 2018, FAO, 2020) [40, 17]. It thrives well in hyposaline water of salinity 0.5 ppt to 10 ppt also and could be culture in coastal region and salty barren land for livelihood security (Ali *et al.*, 2015; Kumar *et al.*, 2017) [6, 29]. More than 1.5 million tonnes of pangasius is produced by Vietnam and traded to over 100 countries worldwide as fillets (Thi *et al.*, 2013; Waycott, 2015) [59, 66].

Looking to favourable cultivable traits of *P. hyphophthalmus* such as tolerate to low dissolved oxygen (Lefevre *et al.*, 2011) [34], fast growth, air breathing habit, and compatibility to polyculture it becomes popular culture species in many Asian countries (Shrestha *et al.*, 2015) [56]. In India it was introduced in 1995-96 for culture (Ahmed and Hasan, 2007; Rao, 2010) [3, 50] then spread very fast and now India becomes one of the largest producers of pangasius through pond cultured system (Rao, 2010) [50]. As it is cultured under high stocking density with the use of huge quantity of feed (Sayeed *et al.*, 2008) [53] so highly vulnerable to disease outbreak.

In pangasius farming, feed cost contributes about 70-90% of the total production cost (Da *et al.*, 2011; Waycott, 2015) [66] may bring loss or narrow profit margin. One of the strategies to improve aquaculture production and sustainability is to focus on enhancing feed nutrient utilization. This may be possible by improving the feed quality and formulating the feeding strategy in such a way that the nutrients can be efficiently delivered and finally utilized. Beneficial effect on growth, survival and body composition of *P. hypophthalmus* using herbs, immunostimulants, enzymes and probiotics have been reported by many researchers. (Labh *et al.*, 2017; Rachmawati and Somidjan, 2018; Khalil *et al.*, 2018; Baleta, 2019; Bala *et al.*, 2020) [31, 48, 28, 12, 11].

Garlic (*Allium sativum*) is famous for centuries may be due to its dietary important for human health and medicinal properties. It contains antimicrobial properties (Harris *et al.*, 2001) [20]. Many researchers recorded that garlic has potent antioxidant and antibacterial properties (Horita *et al.*, 2016; Noda *et al.*, 2013; Yousefi *et al.*, 2020) [21, 41, 68]. Allicin is one of the active components of garlic performs antibacterial activity against disease causing pathogens *Aeromonas*, *Pseudomonas* and *Edvardsiella tarda* (Lee and Yang, 2012) [33]. Higher growth and survival have been evaluated as dietary garlic effect on rainbow trout (Gabor *et al.*, 2012) [18], Nile tilapia (Aly and Mohamed, 2010) [9] and swordfish (Kalyankar *et al.*, 2013) [27]. However, information about garlic effect on pangasius growth, survival, body composition and production were not available. So, to ascertain the same the study has been conducted and observed positive results.

Materials and Methods

Experimental fish and site

Fry of striped catfish *P. hypophthalmus* of mean weight 5.10±0.230 g and length 82.0 ±0.287 mm was collected from Kasad Fish nursery, Kasad, Olpad (Gujarat) and kept in cages of size (2m x 2m x 1.20m) in freshwater pond and fed with basal diet without garlic powder (commercially floating pelleted feed of 30% CP and 4% fat content) thrice a day for seven days before conducting growth experiment. Culture experiment was conducted in carp fish pond at Krishi Vigyan Kendra, Navsari Agricultural University, Navsari-396 450 (Gujarat-India) located near the historic road of Dandi March at altitude of 10m above mean sea level and at Latitude 20.93° North, Longitude 72.89° East.

Experimental diet

Basal diet containing 30-32% crude protein content (commercially available pangasius feed) was used in the experiments (Hung *et al.*, 2004; Silarudee *et al.*, 2019) [23, 57]. Garlic powder inclusive levels 0, 10, 20 and 30 g kg^{-1} feed (Lee *et al.*, 2014; Tiamiyu *et al.*, 2017; Zare *et al.*, 2021) [60, 71] by top dressing the feed using food grade binder were used to be fed to the experimental fish. The treatments of experiment diets were designated as T_1, T_2, T_3 and T_4 for 0, 1, 2 and 3% garlic powder (GP) incorporated diets respectively. The basal diets containing 30 and 32% protein was blended in such a way that final experiment diets of different GP variants became almost iso-nitrogenous and isocaloric.

Whole bulbs of Garlic (*Alium sativum*) were purchased from Sardar Patel APMC, Market, Surat for powder preparation. Cloves were removed from garlic bulbs, peeled out manually and crushed into coarse slices using grinder. Coarse slices of garlic and peels were dried to less than 5% moisture using microwave vacuum drying methods with the support of Centre of Excellence, Food processing Technology, Navsari Agricultural University, Navsari and grinded into fine powder (Yu and Xu, 2007; Erande and Bhambar, 2019) [69, 16]. Garlic powder packed into airtight plastic container and kept in refrigerator for further use in experiments.

Experimental design

Following Complete Randomized Design (CRD) total 4800 fry of the striped catfish *P. hypophthalmus* of mean weight 5.47 ±0.30 g and length 85.20 ±0.84 mm was equally divided into 20 PVC plastic net cages of size (2m x 1m x 1.20m) with stocking density 120 numbers per cubic meter (Rahman *et al.*, 2005; FAO, 2020) [49, 17]. Thus, four treatments of varying level of GP with five replicates were allocated randomly to *P. hypophthalmus*. Fish were fed with different level of garlic powder incorporated diets and basal diet using food grade binder by top dressing during the experiment. Initially they were fed @ 8% of their body weight reducing to 3% towards the end of experiment or satiation thrice daily at 8:00 am in the morning, 1.00 pm afternoon and 5:00 pm in the evening (Silarudee *et al.*, 2019) [57].

Proximate composition of the feed

Proximate compositions such as moisture, crude protein, crude lipid, crude fiber and ash content of feeds and garlic powder were analyzed using standard methods mentioned in AOAC (2005) [1] and calorific or gross energy (kcal/g.) value of the experimental diets was obtained using following formula mentioned by Patel *et al.* (2019) [45].

$$\text{Gross energy (K.cal/g)} = \frac{\text{Crude protein x 5.65 + crude fat x 9.5 + carbohydrate x 4.1}}{100}$$

$$\text{Survival (\%)} = \frac{\text{No. of fish survived after rearing}}{\text{No. of fish stocked}} \text{ x 100}$$

Water quality parameters

Water quality parameters such as temperature, pH, dissolved oxygen (DO), total ammonia and total alkalinity of pond water around experimental units were measured fortnightly following APHA (1985) [10] procedures.

Fish sampling for growth study

About 10 numbers of fish were sampled randomly at every month during rearing period of 180 days and recorded the growth in terms of length (mm) and weight (g) using measuring tape and electronic balance (0.01g accuracy) respectively. After completion of culture period on 181th Day about 50 numbers of fish were selected randomly to measure final length (mm) and growth (g).

Growth parameters

Growth parameters such as final length and weight, net gain in length and weight, percentage final weight, average daily weight and length gain (ADG) and specific growth rate (SGR) were measured using following formulas (Tok *et al.*, 2016) [61].

1. Percentage weight gain (%WG) = (Final weight– initial weight) ÷ (Initial weight) x100.
2. Percentage specific growth rate (%SGR) = (Log$_e$ final body wt. - log$_e$ initial body wt.) ÷ culture days x 100
3. Average daily weight gain (ADG) = (Final weight – initial weight) ÷ Days of culture

Feed conversion efficiency (FCE)

FCE measures the percentage weight of fish produced per unit weight of food consumed. It was calculated using the following formula. (Steffens, 1989; Tok *et al.*, 2016) [58, 61].

$$\text{FCE (\%)} = \frac{\text{Wet weight gain}}{\text{Feed intake}} \text{ x 100}$$

Food conversion ratio (FCR)

It measures the quantity of feed utilized to get unit weight gain in flesh. Thus, it focused on quality of feed and efficiency of fish to convert feed into flesh (USAID, 2011). It was estimated using the formula given below (Steffens, 1989; Tok *et al.*, 2016) [58, 61].

$$FCR = \frac{\text{Feed offered (Dry weight)}}{\text{Body weight gain (wet weight)}}$$

Protein efficiency ratio (PER)

It measures rate of weight gain of fish per unit consumption of protein. PER was calculated using the formula (Steffens, 1989; Tok *et al.*, 2016) [58, 61].

$$PER = \frac{\text{Increment in body weight (g)}}{\text{Protein intake (g).}} \times 100$$

All fish were harvested from each experimental units and counted to measure the final survival. The survival of the fish was estimated using following formula (Tok *et al.*, 2016) [61].

$$\text{Survival (\%)} = \frac{\text{No. of fish survived after rearing}}{\text{No. of fish stocked}} \times 100$$

Yield (Fish production)

Fish were harvested from each experimental unit and kept in plastic crate to estimate yield. Fish yield was measured in kg. Weighed all the fish of each experimental unit using an accurate digital balance.

Body Indices

Body indices may show the percentage of weight of particular organ with respect to total weight of the body (Gireesha, 2002) [19]. Two body indices VSI (visceral somatic index) and HSI (Hepato somatic index) were calculated. On the completion of the experiment, about five fish from each experimental unit were randomly selected and dissected out viscera and liver to estimate body indices.

VSI shows percentage proportional of visceral mass to whole body weight of fish and calculated using following formula (Ighwela *et al.*, 2014) [24].

$$VSI = \frac{\text{Weight of viscera (g)}}{\text{Weight of fish (g)}} \times 100$$

HSI measures the liver condition using following formula (Pandit *et al.*, 2019) [44].

$$HSI = \frac{\text{Weight of hepatopancreas (g)}}{\text{Weight of fish (g)}} \times 100$$

Proximate composition of fish

Proximate compositions of *P. hypophthalmus* were analyzed before commencement of garlic treatments and after completion of the culture experiment to ascertain the changes in the carcass composition of treated groups. Before commencement of the experiment about 400 numbers fish of mean weight 5.47±0.30 g and length 85.20±0.84 mm was used for proximate composition. On completion of the culture period, five fishes were selected randomly from each experimental unit (cage) of different treatments for the same. Dry matter (DM), crude protein (CP), crude fat (EE), and total ash were estimated as per AOAC (2005) [1]. The Nitrogen free extract (NFE) value was derived by subtracting the sum of crude protein, EE and total ash from total dry matter (Woods and Aurand, 1997) [67]. The gross calorific content of the fish was obtained using the multiplying factor 5.65 for protein, 9.5 for fat and 4.1 for carbohydrates as mentioned by Patel *et al.* (2019) [45].

Apparent protein and lipid retention

Apparent value of protein and lipid retention was calculated according to Patel *et al.* (2019) [45] using following formula.

APR (Apparent protein retention) = $(P_2 - P_1) \div Pi$

Where, P_2= Crude protein of the fish at the end of experiment, P_1= Crude protein in the fish at the start of the experiment, Pi= protein intake during the experiment

ALR (Apparent lipid retention) = $(L_2 - L_1) \div Li$

Where, L_2= Crude lipid of the fish at the end of experiment, L1= Crude lipid in the fish at the start of the experiment, Li= Lipid intake during the experiment

Statistical analysis

The data were analysed using SPSS software version - 16 using one way ANOVA and Duncan's multiple range test ($P \leq 0.05$). The data analysis was done at Department of Agricultural Statistics, Navsari Agricultural University, Navsari.

Results and Discussion

Proximate composition of feed

The proximate composition of basal diets and GP was analysed before conducting experiments and results are given in Table 1. Results are in agreement with the published information (Tram *et al.*, 2011; Nguyen *et al.*, 2012) [, 39]. The mean crude protein content of the test diets was in the range of 29.60 – 30.00%, which is in the range of CP (27-32%) required for maximum growth of striped catfish (Hung *et al.*, 2002) [23].

Water quality parameters

As per Table 2. water temperature ranged from 22.1-31.2°C, Dissolved oxygen (4.8-6.8mgL^{-1}), pH (8.1 to 8.4), total ammonia (0.12-0.30mgL^{-1}) and total alkalinity (112-132mgL^{-1}) which fall in suitable range suggested for tropical fish (Waycott, 2015; Devi *et al.*, 2017; Volstorf, 2021) [66, 65].

Growth and survival of fish

Length of *P. hypophthalmus*

Total mean length attained by *P. hypophthalmus* was recorded monthly over the rearing period of 180 days. As per statistical analysis there was no significant difference (P<0.05) in the length up to Day 30. Fig. 1 showed that on Day 60 and onwards higher total length was recorded in GP treated fish than control. Significantly higher (P<0.05) total length 211.80, 269.20, 286.60, 312.00 and 376.80 mm was recorded on Day-60, Day-90, Day-120, Day-150 and Day-180 respectively in T3 (GP2%) groups.

Table 3. showed that GP treated groups showed higher final length, gain in length and average daily gain in length (ADG-L) than control. Significantly higher (P<0.05) final length of 376.8 mm, net gain in length (291.6 mm) and ADG-L (2.06 mm) were observed in T3. However, there was no significant difference between T3 and T4 for above fish length parameters.

In accordance to the above results significantly higher (P<0.05) total length was observed in *Mugil cephalus* (Akbary *et al.*, 2016) [5] and in *Clarias gariepinus* (Nwabueze, 2012) supplemented with GP (0.5, 1.0 and 3.0%) than control.

Results of the present study are almost on similar pattern to Muzaffar *et al.* (2017) [37] who found increasing trend of total length in *Cyprinus carpio* with increasing level of GP inclusion up to GP2% then decreased in GP3%. Moreover, significantly higher (*P*<0.05) length was observed in *Onchorhychus mykiss* (Adineh, 2020) [2] and in yellow croaker, *Larimichthys crocea* (Huang *et al.*, 2020) [22] using encapsulated GP extract (1 and 2%) and allicin (0.005, 0.01, and 0.02%) incorporated diets respectively.

Wet weight and SGR of *P. hypophthalmus*

Mean weight (g) of *P. hypophthalmus* was measured monthly over the rearing period of 180. As per Fig. 2 there was no significant difference (*P*<0.05) in mean weight up to Day 30. But on Day 60 and onwards significantly higher (*P*<0.05) weight was recorded in GP treated groups than control. Higher (*P*<0.05) weight of 101.01,195.10, 377.6, 483.0 and 621.75 g was observed on Day-60, Day-90, Day-120, Day-150 and Day-180 respectively in T3.

As per Table 3 significantly higher (*P*<0.05) final weight, net gain in weight, average daily gain in weight (ADG) and SGR were observed in GP treated groups than control. Significantly higher (*P*<0.05) final weight of 621.75 g, net gain in weight (616.28 g), ADG (3.424g) and SGR (2.63% day^{-}) were found in T3. However, there was no significant difference between T3 and T4 for above growth parameters.

In this study growth in terms of weight and SGR was increased with increasing inclusion of GP in diet up to GP 2% then it was slightly decreased in GP 3%. Thus, study revealed that GP2% is the most effective treatment for enhancing the growth of *P. hypophthalmus*. Higher growth using garlic is advocated because garlic and its peelscontain important prebiotics fructooligosaccharide that can improve gut probiotics (*Lactobacillus acidophilus*) for better digestibility of nutrients and growth (PrayogiSunu *et al.*, 2019) [47]. Moreover, Kumar *et al.* (2010) [30] also mentioned garlic as growth promoter.

Results of the present study are in agreement to others those recorded higher final weight and SGR using garlic in other species such as *O. niloticus* (Shalaby *et al.*, 2006; Aly *et al.* 2008; Metwally, 2009) [55, 8, 36], sterlet sturgeon (Lee *et al.*, 2014), *Mesopotamichthys sharpeyi* (Maniat *et al.*, 2014), Seabass *D. labrax* (Saleh *et al.*, 2015) [51], *Tilapia zilli* (Ajiboye *et al.*, 2016) [4], *Dicentrarchus labrax* (Irkin and Yigit, 2016).

C. carpio (Muzaffar *et al.*, 2017) [37], *Salmo cuspius* (Zaefarian *et al.*, 2017), Amur carp, *Cyprinus carpio haematopterus* (Chesti and Chauhan *et al.*, 2018) [13], Setijaningsih *et al.* 2021) [54], rainbow trout *O. mykiss* (Adineh *et al.*2020) [2] and yellow croaker *L. crocea* (Huang *et al.*, 2020) [22], But Zare *et al.* (2021) [71] did not find any significant (*P*<0.05) growth promoting effect in Eurasian perch *Perca fluviatilis* treated with GP as similar dose of the present study. The negative effects have also been reported. Ndong and Fall (2011) observed decreased growth in hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus)* fed with GP (0.5 and 1.0%) incorporated diet. This may be due to the short treatment period.

In the present study, all dietary GP grouped showed increment in weight over the control but failed to produce any more increment in GP3% treatment. This may be due to negative effect of excess dose of garlic on digestion, liver and kidney at relatively higher dose (Al-Salahy and Mahmoud, 2003) [7]. Similar trend also recorded by Shalaby *et al.* (2006) [55] and Adineh *et al.* (2020) [2].

Feed utilization

As per Table 3 GP treated groups showed higher (*P*<0.05) FCE, PER and lower FCR than non-garlic. However, there was no significant difference between T3 and T4 for the same parameters.

In the present study it is revealed that FCE and PER were increased with increasing level of GP up to GP 2% then slightly decreased in GP 3%. While in case of FCR inverse trends of decreased FCR with increasing level of GP up to 2% was observed then slightly increased. Similarly, pattern in *O. niloticus* was observed by Shalaby *et al.* (2006) [55]. In accordance to present study significantly (*P*<0.05) improved FCE, PER and FCR were recorded in Sterlet sturgeon (Lee *et al.*, 2014), in *O. mykiss* (Adineh*et al.*, 2020) [2], in *T.zilli* (Ajiboye *et al.*, 2016) [4] in *O. niloticus* (Metwally, 2009) [36], Amur carp (Chesti and Chauhan, 2018) [13], *S. cuspius* (Zaefarian *et al.*, 2017), *M. sharpeyi* (Maniat *et al.*, 2014), red tilapia *Oreochromis* sp. (Samson, 2019) and *D. labrax* (Saleh *et al.*, 2015) [51] fed with dietary garlic. But Zare *et al.* (2021) [71] did not find significant (*P*<0.05) effect on FCR of *P.fluviatilis.*

Fish yield

Table 3 showed that fish yield ranged from 115.55- 146.23 kg and GP treated groups have higher yield than non-GP treated. Significantly higher yield of 146.23 kg was observed in T3 which is about 27% higher than T1. However, there was no significant (*P*<0.05) difference between T2 and T4. Coincides with the result of the present study Muzaffar *et al.* (2017) [37] also found higher (*P*<0.05) yield of *C. carpio* supplemented with dietary GP 2% for 120 days.

Survival

At the end of experiment survival was estimated. Table 3 shows that significantly higher (*P*<0.05) survival of 98% was observed in T3 while lower (95.50%) in T4. Statistically, T3 and T1 are at par whereas T2 and T4 did not show any significant difference. Study reveals that though higher survival was observed in GP2% but no distinguished effect of GP on survival was noticed. Partial agreement to the present study no significant difference (*P*<0.05) in survival rate were notice in garlic treated groups of fish such as *A. ruthenus* (Lee *et al.*, 2014), *M. sharpeyi* (Maniat*et al.*, 2014), *O. mykiss* (Adineh*et al.*, 2020) [2], *P.fluviatilis* (Zare*et al.*, 2021) [71] and in *C. carpio* (Muzzafar*et al.*, 2017). However higher (*P*<0.05) survival rate in *O. niloticus* (Aly *et al.*,2008) [8], in red tilapia (Samson, 2019), in *L.crocea* (Huang *et al.*,2020), in *Tilapia zilli* (Ajiboye*et al.*, 2016) [4] and *O. niloticus* (Metwally, 2009) [36] was found in garlic treated groups than control.

Body indices

Viscerosomatic index (VSI) and Hepatosomatic index (HSI)

As per Table 4. GP (1-3%) treated fish showed significantly lower VSI and HSI than control. Higher (*P*<0.05) VSI of 15.06 and HSI (2.06) were recorded in T1 while lower (*P*<0.05) VSI of 13.29 and HSI of 1.93 were observed in T3. However, all GP treated groups did not differ significantly (*P*>0.05) in VSI whereas T2 and T4 are statistically at par for HSI.

In accordance to present study lower VSI and HSI were recorded in GP treated fish such *O. mykiss* (Adineh *et al.*,

2020) [2], *O. niloticus* (Metwally, 2009) [36] and *S. cuspius* (Zaefarian *et al.*,2017). But GP treated groups of *P.fluviatilis* (Zare*et al.*, 2021) [71] and *O. niloticus* (Shalaby *et al.*, 2006) [55] did not show any significant (*P*<0.05) changes in HSI and VSI.

Bio-chemical composition

Proximate composition of *P. hyphophthalmus* such as dry matter (DM), crude protein (CP), crude lipid or ether extraction (EE), total ash and nitrogen free extract (NFE) were analysed before and after completion of garlic trials. As per Fig. 3 GP treated groups showed significantly higher lost in moisture than control. Higher (*P*<0.05) lost in moisture about 33% was recorded in GP2% treated group. As far as final dry matter DM and crude protein CP are concerned GP treated groups showed higher content than control (Fig.4&5). Significantly higher (*P*<0.05) DM of 45.98% and CP (24.47%) were observed in T3. Inverse to above Fig. 6 and 7 illustrated that all GP treated groups have lower final crude lipid and ash than control. Significantly higher (*P*<0.05) crude lipid of 21.72% and ash (2.95%) were analysed in T1 while lower (*P*<0.05) lipid (14.74%) and ash (2.12%) were observed in T3.

Fig 4 & 5 illustrating higher gain in DM and CP in all GP treated groups than control. Significantly higher (*P*<0.05) gain in DM and CP of 151.55% and 125.98% were recorded in T3 respectively. In contrast to this fig 6&7 showed lower gain in crude lipid and ash in GP treated groups than control. Significantly lower (*P*<0.05) gain in lipid of 386.54% and ash (5.90%) were estimated in T3. Whereas higher (*P*<0.05) for the same in T1. Thus, lipid lowering effect and improvement in protein content of *P. hyphophthalmus* was noticed in GP treated groups. GP2% emerged as more effective dose than others.

As per Fig. 8 no significant effect on NFE was observed. Due to lower fat content in GP treated groups significantly lower calorific value of (2.98-3.04 kcal/g.) was observed in T3 while higher (3. 13kcal.g) was recorded in T1 (Fig. 9). Thus, it is suggested that with the use of GP2% high proteaceous low-calorie *P. hyphophthalmus* can be produced and may improve quality of fillets and storage due to lower fat content.

In agreement to the findings of the present study significantly higher (*P*<0.05) Dry matter (DM) was recorded in *A. ruthenus* (Lee *et al.*,2014) in *S. caspius* (Zaefarian *et al.*,2017) andin *O. mykiss* (Adineh *et al.*, 2020) [2] fed with GP incorporated diets. In contrast to above results Ajiboye *et al.* (2016) [4] and Shalaby *et al.* (2006) [55] observed significantly lower DM in Monosex *Tilapia zillii* and *O. niloticus* treated GP. While Zare *et al.* (2021) [71] and Metwally (2009) [36] did not find significant difference in DM of Eurasian Perch *P. fluviatilis* and *O. niloticus* respectively treated with garlic.

Coinciding with results of the present study higher (*P*<0.05) CP and lower (*P*<0.05) lipid using garlic was observed in in *A. ruthenus* (Lee *et al.*, 2014) *T. Zillii* (Ajiboye *et al.*, 2016) [4], *O. niloticus* (Shalaby *et al.*, 2006) [55], *M. sharpeyi* (Maniat *et al.*, 2014), *O. mykiss* (Adineh *et al.*, 2020) [2] and in *D. labrax* (Saleh *et al.*, 2017; Irkin and Yigit, 2016). Whereas, Zare*et al.* (2021) [71] observed higher (*P*<0.05) CP but no significant change in lipid in *P. fluviatilis* supplemented with GP 3%. But partial agreement to present study Huang *et al.* (2020) [22] observed significantly (*P*<0.05) lipid lowering effect only but not change in protein in large yellow croaker *L. crocea* using dietary allicin.

In accordance to present findings lower (*P*<0.05) ash recorded in *A. ruthenus* (Lee *et al.*, 2014) [32], Monosex *Tilapia zillii* (Ajiboye*et al.*, 2016) [4], *O. mykiss* (Adineh *et al.*, 2020) [2], *P. fluviatilis* (Zare *et al.*, 2021) [71] and in *O. niloticus* (Metwally, 2009) [36] fed with garlic. Similar to some extend Irkin and Yigit (2016) found lower gross energy in European seabass (*D. labrax*) juveniles treated with garlic meal incorporated diet than control.

Apparent protein and lipid retention

As per Table 4 higher (*P*<0.05) apparent protein retention (APR) and apparent lipid retention (ALR) were observed in all garlic treated fish than non-garlic one. Significantly higher (*P*<0.05) APR (0.686) and ALR (6.212) were estimated in T3 However, there was no significant difference in T2 and T4.

In accordance to present study higher (*P*<0.05) APR and ALR were confirmed in fingerling of sterlet sturgeon, *A. ruthenus* (Lee *et al.*, 2012) fed with GP 0.5% diet and in fry of *C. gariepinus* (Nyadjeu *et al.*, 2021) [43] supplemented with GP1% incorporated diet. Similarly, to the result of present study Lee *et al.* (2014) observed higher (*P*<0.05) APR in juveniles of *A. ruthenus* but in contrast to the present study found lower (*P*<0.05) ALR in the same with GP treatments.

Table 1: Proximate composition of basal diets and GP (% DM basis Mean ±S. E)

Types of feed	Moisture (%)	DM (%)	Crude protein (%)	Ether extract (%)	Fibre (%)	Ash (%)	NFE (%)	Calorific value (Kcal/g)
Commercial pangasius feed (30% Protein)	8.08 ±0.007	91.92 ±0.007	29.6 ±0.138	4.11 ±0.068	4.13 ±0.214	18.43 ±0.174	35.65 ±0.104	3.69 ±0.046
Commercial pangasius feed (32% Protein)	8.40 ±0.123	91.60 ±0.123	31.8 ±0.087	4.08 ±0.091	4.10 ±0.186	18.60 ±0.150	33.02 ±0.166	3.71 ±0.049
Garlic powder	4.12 ±0.150	95.89 ±0.150	19.51 ±0.03	0.55 ±0.026	1.85 ±0.082	3.9 ±0.075	70.08 ±0.243	4.1 ±0.058

DM- Dry matter; NFE- Nitrogen free extracts

Table 2: Physico-chemical parameters of pond water

Water quality parameters	Range		Mean ±S.E.
	Minimum	Maximum	
Temperature (°C)	22.1	31.2	27.71 ±0.849
Dissolved oxygen (mgL^{-1})	4.8	6.8	5.76 ±0.163
pH	8.12	8.4	8.34 ±0.022
Total Alkalinity (mgL^{-1})	112	132	123.46 ±0.998
Total ammonia(mgL^{-1})	0.12	0.30	0.2 ±0.016

Table 3: Growth parameters, feed efficiency, yields and survival of *P. hypophthalmus* supplemented with garlic. Mean ±S. E.

Parameters	0% Garlic (T1 control)	1 % Garlic (T2)	2 % Garlic (T3)	3% Garlic (T4)	SEM	*P* Value	Test
Initial length(mm)	85.2 ±0.374	85.2 ±0.374	85.2 ±0.374	85.2 ±0.374	0.374	1.00	*NS*
Initial weight (g)	5.47 ±0.136	5.47 ±0.136	5.47 ±0.136	5.47 ±0.134	0.136	1.00	*NS*
Final length (mm)	351.8 ±6.85^{c}	360 ±2.28^{bc}	376.8 ±2.13^{a}	365.6 ±2.62^{ab}	3.99	0.003	**
Final weight (g)	498.49 ±10.98^{c}	547.01 ±9.89^{b}	621.75 ±8.88^{a}	566.14 ±14.56^{b}	11.280	0.001	**
Net gain length (mm)	266.6 ±6.66^{c}	274.8 ±2.13^{bc}	291.6 ±2.06^{a}	280.4 ±2.77^{ab}	3.9	0.003	**
Netgain weight (g)	493.02 ±10.89^{c}	541.54 ±9.79^{b}	616.28 ±8.78^{a}	560.67 ±14.63^{b}	11.24	0.001	**
ADG-length (mm)	1.92 ±0.037^{c}	1.97 ±0.011^{bc}	2.06 ±0.012^{a}	1.99 ±0.015^{ab}	0.063	0.003	**
ADG- weight (g)	2.739 ±0.061^{c}	3.01 ±0.054^{b}	3.424 ±0.049^{a}	3.12 ±0.081^{b}	0.063	0.001	**
SGR (%)	2.51 ±0.012^{c}	2.56 ±0.0.01^{b}	2.63 ±0.0.009^{a}	2.58 ±0.024^{b}	0.014	0.001	**
(%) WG	49305.4 ±239.20^{d}	54318.32 ±445.25^{c}	61850.4 ±534.36^{a}	56774.38 ±311.74^{b}	399.44	0.001	**
FCE (%)	63.96 ±1.41^{c}	70.26 ±1.27^{b}	79.95 ±1.14^{a}	72.74 ±1.90^{b}	1.459	0.001	**
FCR	1.56 ±0.034^{a}	1.42 ±0.027^{b}	1.25 ±0.017^{c}	1.38 ±0.036^{b}	0.028	0.001	**
PER	2.16 ±0.048^{c}	2.37 ±0.042^{b}	2.7 ±0.038^{a}	2.45 ±0.063^{b}	0.049	0.001	**
Yield (Kg)	115.55 ±2.77^{c}	125.38±2.28^{b}	146.23±2.11^{a}	130.17 ±3.00^{b}	2.56	0.001	**
Survival (%)	96.58 ±0.847^{ab}	95.50 ±0.082^{b}	98.00 ±0.332^{a}	95.83 ±0.321^{b}	0.48	0.01	*

ADG- Aggregate daily gain; SGR- Specific growth rate; WG- weight gain; FCE- Food conversion efficiency; FCR- Food conversion ratio; PER- Protein efficiency ratio; ** -Highly significant (P<0.01), * - Significant (P<0.05) and *NS* – Non-significant; Means with different superscripts in a column differ significantly

Table 4: Body index and Apparent protein and lipid retention in *P. hypophthalmus* supplemented with garlic powder Mean ±S. E.

Exp. groups	APR	ALR	VSI	HSI
0% garlic (T1)	0.326 ±0.01^{c}	4.982 ±0.11^{c}	15.06±0.12^{a}	2.36 ±0.035^{a}
1% garlic (T2)	0.448 ±0.01^{b}	5.466 ±0.099^{b}	13.64 ±0.42^{b}	2.14 ±0.062^{b}
2% garlic (T3)	0.686 ±0.01^{a}	6.212 ±0.09^{a}	13.29 ±0.14^{b}	1.93 ±0.054^{c}
3% garlic (T4)	0.48 ±0.014^{b}	5.658 ±0.145^{b}	14.06 ±0.34^{b}	2.06 ±0.028^{bc}
SEM	0.014	0.113	0.284	0.047
P Value	0.001	0.003	0.003	0.001
Test	**	**	**	**

APR- Apparent protein retention; ALR- Apparat lipid retention; VSI- Viscero somatic index; HSI- Hepato somatic index; ** -Highly significant (P<0.01), * - Significant (P<0.05) and *NS* – Non-significant; Means with different superscripts in a column differ significantly

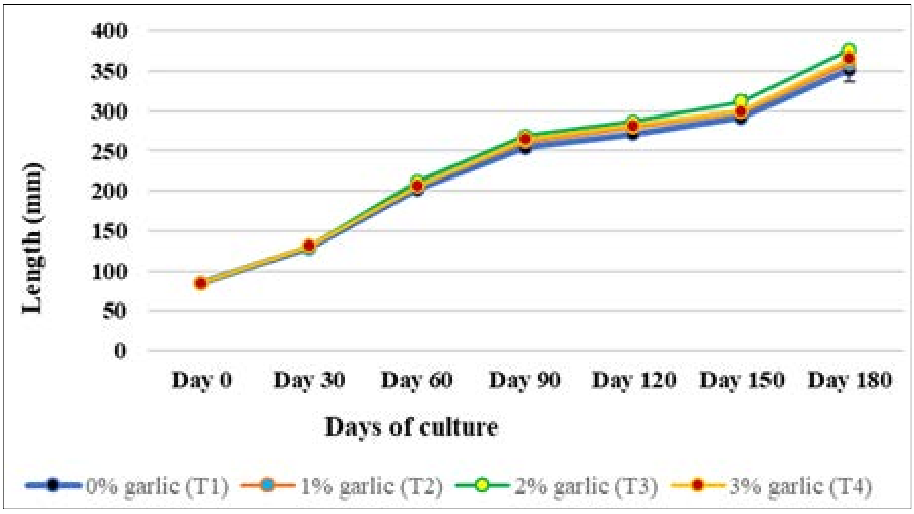

Fig 1: Trends of growth (length) at various period in *P. hypophthalmus* supplemented with garlic (Mean ±S.E.)

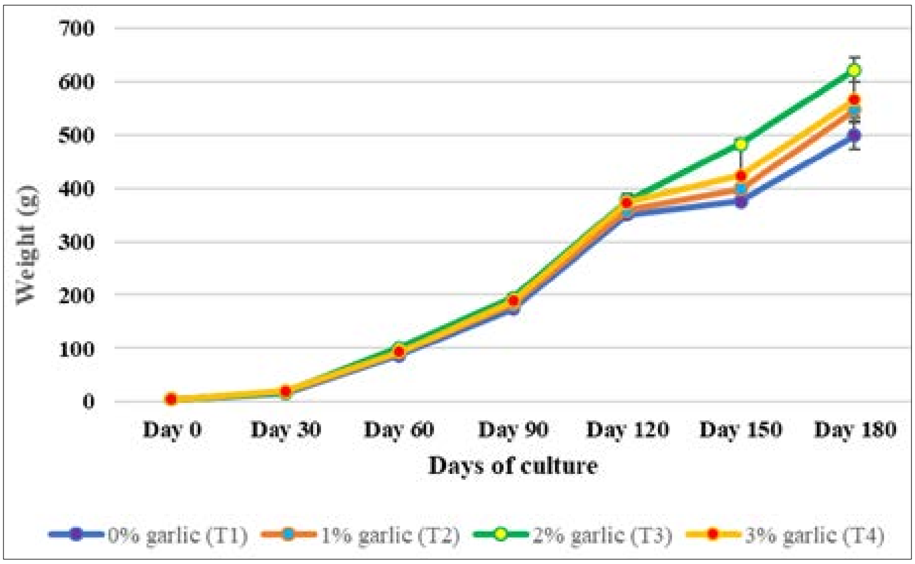

Fig 2: Trends of growth (weight) at various period in *P. hypophthalmus* supplemented with garlic (Mean ±S.E.)

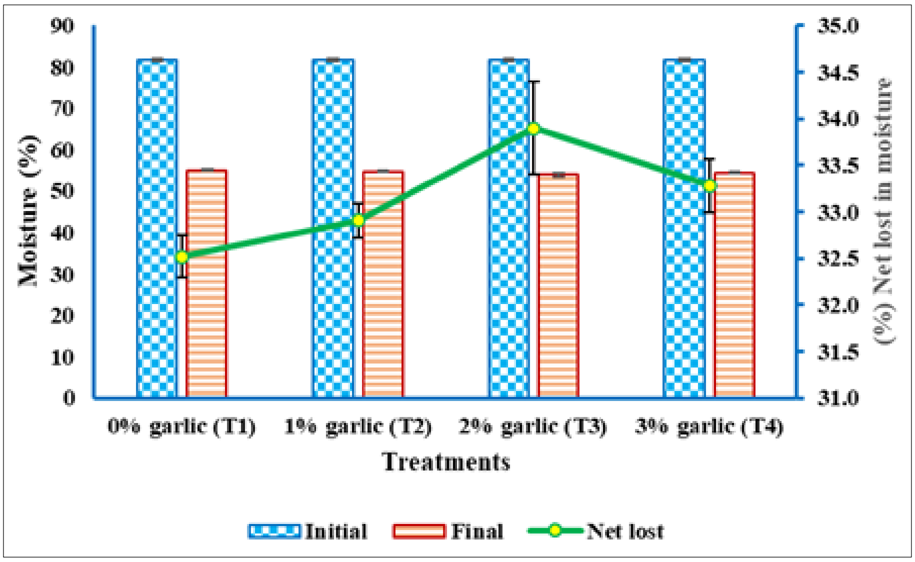

Fig 3: Initial, Final and net lost in moisture of the P. hypophthalmus supplemented with garlic (Mean ±S.E.)

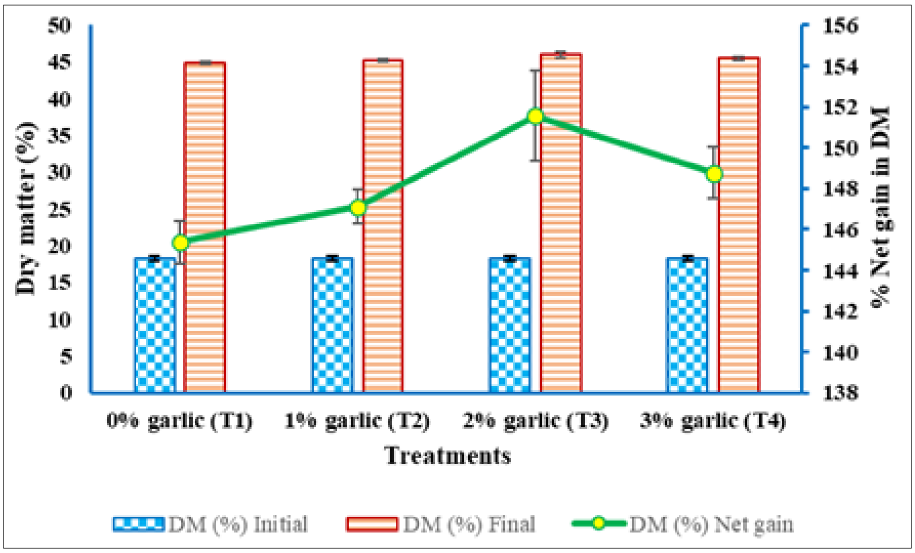

Fig 4: Initial, Final and net gain in Dry matter (DM) of the *P. hypophthalmus* supplemented with garlic (Mean ±S.E.)

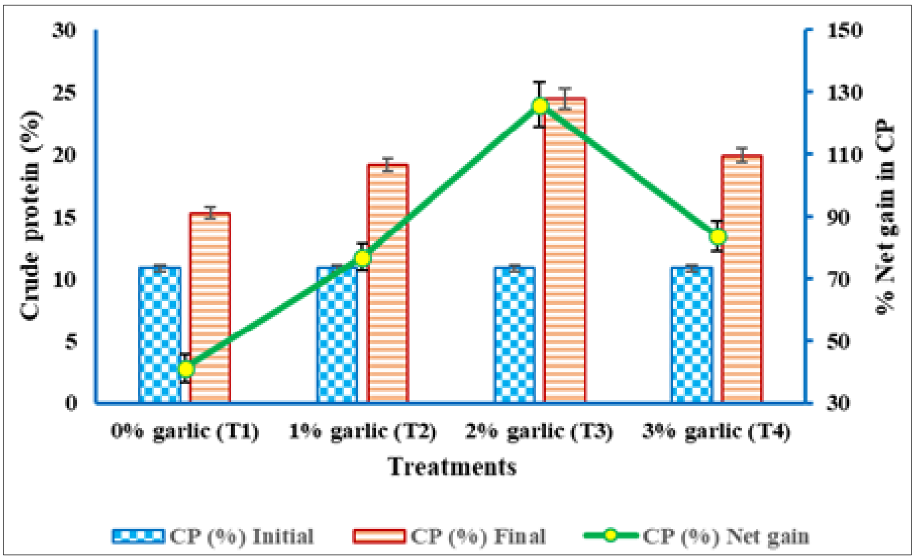

Fig 5: Initial, Final and net gain in Crude protein (C.P.) of the P. hypophthalmus supplemented with garlic (Mean ±S.E.)

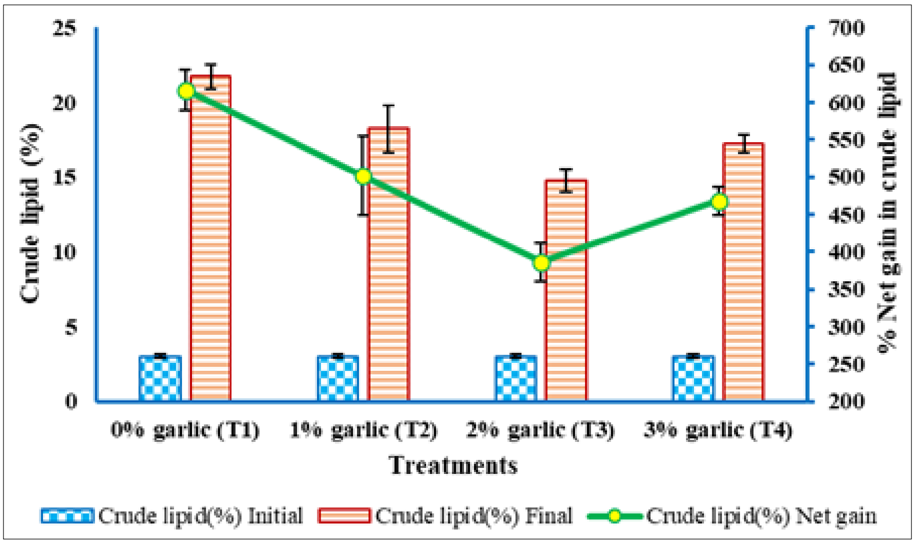

Fig 6: Initial, Final and net gain in Crude lipid of the P. hypophthalmus supplemented with garlic (Mean ±S.E.)

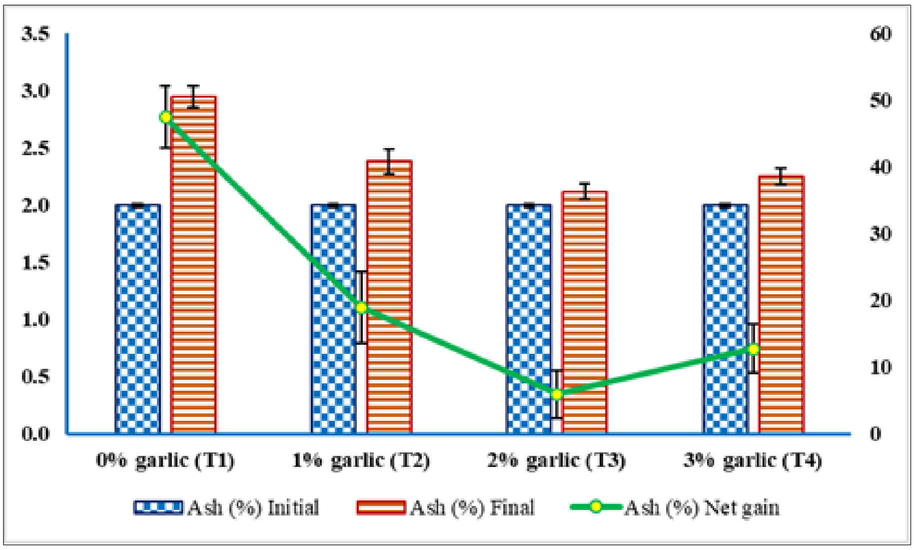

Fig 7: Initial, Final and net gain in Nitrogen free extracts (NFE) of the P. hypophthalmus supplemented with garlic (Mean ±S.E.)

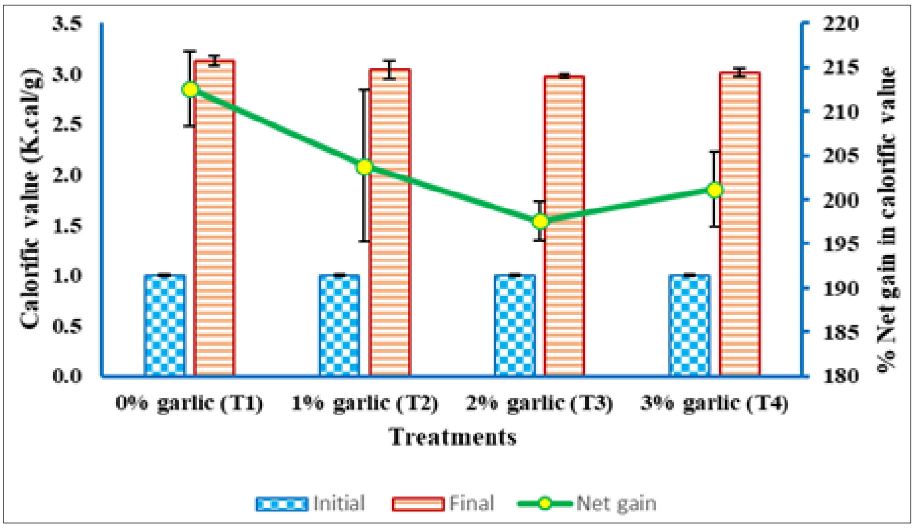

Fig 8: Initial, Final and net gain in Calorific value of the P. hypophthalmus supplemented with garlic (Mean ±S.E.)

Conclusion

Study reveals that *P. hypophthalmus* supplemented with GP at 2% dose for long period not only enhance growth and nutrients digestibility but improves flesh quality (body composition) of *P. hypophthalmus* by imparting lipid lowering and protein improving effect.

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