Indian J. Anim. Nutr. 2022. 39 (4): 512-522
doi: 10.5958/2231-6744.2022.00057.3

Indian Journal of Animal Nutrition

Garlic Powder Supplementation: Effects on Nutrient Utilization and Haematological Parameters of Sutchi catfish (*Pangasianodon hypophthalmus*, Sauvage, 1878)

P. P. Patel[1], R. V. Borichangar[1], V. R. Patel[2] and S. C. Timbadia[1]
[1] College of Fisheries Science, Kamdhenu University, Navsari -396450, Gujarat, India
[2] College of Veterinary Science & Animal Husbandry, Kamdhenu University, Navsari -396450, Gujarat, India
*Correspondence: vrpatel@kamdhenuuni.edu.in

ABSTRACT

The experiment was conducted to evaluate the effect of garlic supplementation on nutrients utilization and haematological assay on Sutchi catfish (*Pangasianodon hypophthalmus, sauvage,* 1878). In first part of experiment total 500 number of *P.hypophthalmus* fingerlings with mean body weight (BW) of 27.74 ±1.46g and 134 ±1.41 mm of length (l) were distributed in four dietary treatment group with five replica in each as T1, T2, T3 and T4, respectively for a period of 60 days to access the nutrient utilization. In second part of the study, the same number at the *P.hypophthalmus* juveniles with 202.67 ±10.32 g BW and 270 ±4.27 mm were reared for 45 days to evaluate haematological parameters. In both the parts of the study the experimental fishes were supplemented with garlic (*Allium sativum*) powder (GP) in diet at the rate of 0, 1, 2 and 3%, respectively and reared in FRP tanks of 100 lit capacities for respective periods. Results revealed that significantly higher ($P<0.05$) feed assimilation efficiency (FAE), apparent digestibility co-efficient (%, ADC), nitrogen utilization (%) and digestible energy (kcal/g) was observed in T3 group supplemented with 2% GP. Significant changes in major blood biochemical and haematological parameters were observed on 45thday of experiment, where higher ($P>0.05$) values were observed for red blood cells (RBC), thrombocytes, packed cell volume (PCV), and haemoglobin (Hb) and lower ($P<0.05$) at 2% GP supplementation, however PCV and mean corpuscular value (MCV) were found higher at 3% GP supplementation (T4 group). No significant effect ($P>0.05$) of GP supplementation was revealed for monocyte, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC). It was concluded that 2% GP supplementation improves the feed utilization and health of Sutchi catfish.

Key Words: Digestibility, Garlic, Haematological Parameters, Nutrient utilization, Sutchi Catfish.

Article received: 21 November 2022; Article accepted: 23 December 2022

INTRODUCTION

Sutchi catfish (*Pangasianodon hypophthalmus*) commonly called as Pangasius, is commercially important fast growing freshwater catfish member of *pangasidae* family (Jeyakumari et al., 2016). Considering favourable cultivable traits of Sutchi cat fish such as tolerate hypo-saline water at 0.5 to 10 ppt salinity (Kumar et al., 2017), low dissolved oxygen (Lefevre et al., 2011), fast growth rate, air breathing habit and compatibility to polyculture and has potential to culture at coastal region and salty barren land for livelihood security (Shrestha et al., 2015).In India, it was introduced for culturing in the year of 1995-96, then it becomes widely spread and now India emerges as one of the largest producers of pangasius through pond cultured system (Rao, 2010).

To achieve the target of economical and higher production, there isimprudentuse of antibiotics and other feed additives in aquaculture. Their residues become harmful to environment and food safety concern, which enforced consumers and researchers to switchover organic or herbal food production in the modern world. Many phyto addtives have growth promoting and immuno-stimulating properties, offering economical and eco-friendly alternative for health management. Among potential medicinal herbs, garlic (*Allium sativum* L.), belongs Liliaceae

family, is used as spice and also as traditional medicine since long. It is one of the natural immuno-stimulants (Lewis et al., 2003), Moreover, researchers have found that garlic has a many biological functions, such as anti-microbial, anti-inflammatory, anti-atherosclerotic, anti-diabetic, anti-mutagenetic, anti-carcinogenic, antioxidant and immune-modulation activities (Cullen et al., 2005; Kim et al., 2013).Phytochemical analysis showed that the principle compound in garlic is allicin, which is the most powerful sulphur containing non-protein amino acid present in garlic that actively kills parasites and pathogenic bacteria (Adler and Holub, 1997); Many researchers have established that the garlic have potential to eliminate principal pathogenic bacteria, uplift health status, and improve flesh quality in freshwater but still the information on efficacy and appropriate dose of garlic in *P.hypophthalmus* on nutrient utilization and bold-biochemical parameters is inadequate.In this context, the present study was planned to assess the possibility of utilizing garlic powder (GP) as feed additive for improving nutrient utilization and health status of *P.hypophthalmus*.

MATERIALS AND METHODS

Experimental fish and site

In order to ascertain the effect of dietary garlic on nutrients utilization and haematological parameters the study was conducted in at KrishiVigyan Kendra, Navsari Agricultural University, Navsari-396 450 (Gujarat-India) in two separate experiments. Fingerlings of *P. hypophthalmus* of mean weight 27.74 ±1.46 g and length 134 ±1.41 mm was used to evaluate digestibility for a period of 60 days and juveniles of mean weight 202.67 ±10.32 g and length 270 ±4.27 mm was used to study haematological parameters for a period of 45 days. Since the fingerlings are smaller in size and delicate than juveniles, in order to drawn sufficient amount of blood without mortality of fish juveniles of about 200 g were used for haematological study.

Experimental diet and management

Commercially available pangasius feeds procured from I. B. PVT., Ltd; labelled with 30 (estimated 29.60 ±0.138) and 32% crude protein (estimated 31.80 ±0.087) were used as basal diets as pangasius requires 29-32% CP for higher growth (Hung et al., 2002 and Silarudee et al., 2019) and blended as per described in Table 1, so that final experiment diets of different garlic variants became iso-nitrogenous and isocaloric.(Hung et al., 2004 and Silarudee et al., 2019). The whole bulb of garlic (*Allium sativum*) was purchased from open market and processed in the Food Processing Laboratory at Centre of Excellence, Food processing Technology, NAU, Navsarito make garlic powder (GP). After removing hulls, it was driedthrough vacuum drying methods then grinded along with hulls till to pass through 1 mm screen and stored under refrigerated temperature for further use. The GP was incorporated at the level of 0, 1, 2 and 3% of ration by top dressing the feed using food grade binder.

Table 1. Blending of basal diets for isonitrogenous and isocaloric experimental diets

Treatments	Blending level of pangasius feed (%)		Inclusion level of garlic powder (%)	Final estimated crude protein (%) of blended test diet	Final Calorific value (kcal/g)
	A type	B type			
0% garlic (T1-control)	100	0	0	29.6 ±0.32	370.6 ±1.32
1% garlic (T2)	94.05	4.95	1	29.6 ±0.27	369.8 ±1.08
2% garlic (T3)	88.20	9.80	2	29.6 ±0.18	370.3 ±1.32
3% garlic (T4)	63.05	33.95	3	30.0 ±0.16	371.0 ±0.68

A type feed: commercial pangasius feed labelled with 30% CP, B type feed: commercial pangasius feed labelled with 32% CP

Fingerlings and juvenile of *P. hypophthalmus* each of five hundred numbers were divided equally into four dietary treatment groups, respectively with five replicates in each, therefore twenty FRP tanks of 100 L capacity were used with stocking density twenty-five fish per tank (Biswas et al., 2016) in both the experiments. In both the experiments, fish were fed with garlic incorporated diets as well as control diet @ 3-4% of body weight or satiation at 8:00 am in the morning, 1.00 pm afternoon and 5:00 pm in the evening using food grade binder (Gilannejad et al., 2019; Silarudee et al., 2019).

Digestion trial

Total five hundred fingerlings of *P. hypophthalmus* divided equally into twenty FRP tanks of 100 L capacity with stocking density twenty-five fish per 100 L FRP tank (Biswas et al., 2016). Thus, four treatments of garlic incorporated experimental diets (T1-0%, T2-1%, T3-2% and T4-3%) with five replicates were allocated randomly to *P. hypophthalmus* stocked in FRP tanks following the Completely Randomized Design (CRD).Fish were acclimatized to indoor laboratory condition in FRP tanks for two weeks prior to the study and fed experimental diet to satiation level of 5% of the body weight using sprinkle method.Ammonia excretion rate of fish was estimated as per method described by Yigit et al. (2003) and Rafiee et al. (2006) before commencement of the experiment.

During digestion trial the faeces was collected as soon as fish defecate in morning before feeding and late evening for 60 days of collection period following direct or conventional method (De la Noue and Choubert, 1986; Zewdie, 2019) where the water was siphoned using sieve of nylobot cloth of 200 microns (Patel et al., 2019).Half of the faeces was preserved with few drops of concentrated sulphuric acid for nitrogen estimation and remaining was oven dried at 60°C and both were pooled as per replica then stored at -20°C for further analysis (Tu-Tran et al., 2020).

Feed and faeces were analysed for its proximate compositions as per AOAC (2005).Apparent nutrients digestibility co-efficient indicates the percentage value of nutrients absorbed with respect to ingested nutrients which was calculated as per Paulraj and Easterson (1982). While digestible energy (kcal/g) was measured by subtracting gross energy of faeces from the gross energy of ingested feed according to Sawant et al. (2003) and Hien et al. (2010). Net protein utilization (NPU) was measured by the multiplication of digestion coefficient of protein (protein digestibility) with biological value (percent of absorbed protein retained in body) as suggested by Zewdie (2019).

Haematological parameters

Prior to conduct experiment, fishes were disinfected using $KMnO_4$at the rate of 2.0 mg L^{-1} for two hours (Darwish et al., 2009) and 1% NaCl for 20 min and acclimated to indoor laboratory condition in FRP tanks for one week. A water quality was maintained by removing faecal matter and exchanging the water up to 50%. Two fish were randomly collected from each replica on 45th day for blood collection to be used for haematological assay. About 1 ml blood was drawn from caudal vein with the help of 20-gauge needle and heparinized hypodermic syringe (2 mL) and placed in anti-coagulant vial (10% EDTA; AcCuvet-Plus; K_3EDTA) and stored at 4°C for estimating haematological parameters as mentioned by Naveenkumar et al.(2017).

Counts of Red Blood Cells (RBC) and White Blood Cells (WBC) were determined using improved Neubauer haemocytometer and calculated (Blaxhall and Daisley 1973). Haemoglobin (Hb g/dL) was estimated using Sahli'shaemoglobinometer. Haematocrit (HCT) value was determined using micro haematocrit capillaries filled with blood and centrifuged at 8,700×g for 5 min and expressed as percentage of total blood volume (Wintrobe, 2008). Mean corpuscular volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) were calculated from the average values of Hb (Dacie and Lewis, 1984).

Statistical Analysis

The data generated were analyzed for their statistical

significance using statistical package for the social sciences (SPSS, version 20.0 Chicago, USA). Data were analyzed using one-way ANOVA to distinguish the impact of different dietary treatments. The effects were considered to be significant at ($P<0.05$) and declared as trend/tendency at $0.05<P<0.10$.

RESULTS AND DISCUSSION

The proximate composition of basal diets and GP was analysed before conducting experiments. The mean crude protein content of the test diets was 29.60 ±0.138 %, which is in the range of CP (29-32%) required for maximum growth of Sutchi catfish (Hung et al., 2002). Appreciable quantity of CP (19.51 ±0.03%) and EE (2.55 ±0.026) was found in garlic powder which was almost similar to that of proximate composition of *Alium sativum* bulb reported by Ali and Ibrahim (2019).Water temperature ranged from 26-27°C, Dissolved oxygen(5.6-6.8mgL-1),pH (8.1 to 8.4), total ammonia (0.05-0.08mgL-1) and total alkalinity (148-152mgL-1) which fall in suitable range suggested for tropical fish (Volstorf, 2021)

Digestibility and assimilation of nutrients

The digestibility of CP, EE, CF, NFE and total carbohydrate was significantly higher in GP treated groups than control (Table 2). Amongst supplemented groups, the nutrient digestibility was found highest at 2% GP supplemented group (T3), the values being 93.5,93.8, 92.0,13.4 and 84.0%, respectively for CP EE, NFE, CF and total carbohydrates, respectively. FAE (%) was increased ($P<0.05$) in GP supplemented groups (Table 1), where highest improvement was observed in T3 (85.32%) followed by T2 (84.22%) and T4 (83.76%). *P. hypophthalmus* supplemented with GP showed higher feed assimilation efficiency, nutrients utilization and DE than without garlic which could be associated with appetite promoting effect by bioactive components of garlic. Digestion and nutrient utilization of the food mainly depends upon size of fish, gut biota, digestive enzymes, appetite regulating hormones and genes (Dabrowski, 1986). Among the groups 2% inclusion of GP in basal feed is the most effective on feed digestion and nutrients utilization. Huang et al. (2020) reported that higher expression of appetite stimulating genes like neuro peptide Y (npy) and ghrelin in large yellow croaker (*L. crocea*) supplemented with allicin (0.01%). Similarly, Esmaeili et al. (2017) observed that increasing the activities of digestive enzyme like trypsin in rainbow trout supplemented with GP. Xu et al. (2020) also observed elevated digestive enzyme activities including trypsin, amylase (AMS) and lipase (LPS) in Japanese seabass, *Lateolabrax japonicus* fed with dietary GP.

Table 2. Feed assimilation efficiency, digestibility of nutrients with nitrogen utilized and digestibility energy of *P. hypophthalmus* supplemented with garlic

Treatments	Feed assimilation efficiency (%)	Digestion co-efficient of nutrients or Digestibility of nutrients (%)					Nitrogen utilized (%)	Digestible energy (k.cal/g)
		Protein	Fat	NFE	Fibre	Carbohydrate		
0% garlic (T1 - control)	83.14 ±0.161^{c}	90.08 ±0.468^{c}	87.14 ±0.642^{d}	89.26 ±0.449^{c}	10.87 ±0.419^{b}	81.12 ±0.441^{c}	85.28 ±0.471^{c}	3.17 ±0.007^{c}
1% garlic (T2)	84.22 ±0.183^{b}	92.17 ±0.262^{b}	91.38 ±0.399^{b}	90.40 ±0.266^{b}	12.01 ±0.491^{ab}	82.35 ±0.25^{b}	87.96 ±0.257^{b}	3.25 ±0.006^{b}
2% garlic (T3)	85.32 ±0.129^{a}	93.58 ±0.207^{a}	93.81 ±0.581^{a}	92.06 ±0.155^{a}	13.44 ±0.681^{a}	84.08 ±0.167^{a}	89.84 ±0.21^{a}	3.31±0.004^{a}
3% garlic (T4)	83.76 ±0.189^{b}	91.99 ±0.219^{b}	88.82 ±0.552^{c}	90.66 ±0.296^{b}	11.53 ±0.583^{b}	82.72 ±0.297^{b}	87.55 ±0.217^{b}	3.25 ±0.006^{b}
SEm±	0.167	0.308	0.552	0.329	0.553	0.306	0.309	0.004
P value	0.001	0.003	0.001	0.001	0.029	0.001	0.001	0.001
Test	**	**	**	**	*	**	*	**

NFE-Nitrogen free extracts; ** - significant at$P<0.01$, * - Significant at $P<0.05$ and *NS* – Non-significant
Means with different superscripts in a column differ significantly

In accordance to the results of present study, higher (P<0.05) apparent digestibility of nutrients was recorded with garlic supplementation in various species by many researchers such as *O. niloticus* (Shalaby et al.,2006), *Tilapia jilli* (Jegede, 2012), rainbow trout (Esmaeili et al.,2017), *Percafluviatilis* (Zare et al.,2021)and *Labeorohita* (Kaur and Ansal, 2020).Higher digestibility of nutrients through GP application in the present study may be advocated because garlic and its peels contain important prebiotics as fructo oligosaccharide that can improve gut probiotics (*Lactobacillus acidophilus*) for better digestibility of nutrients (Prayogi Sunu et al., 2019). Moreover, in agreement to present study Shalaby et al. (2006) and Esmaeili et al. (2017) recorded higher (P<0.05) digestibility energy in *O. niloticus* and rainbow trout treated with GP. In reflection to protein digestion and utilization, significantly higher (P<0.05) nitrogen utilized rate of 89.84% was observed in T3, whereas lower 85.28% was recorded in T1. However, there was no significant difference between T2 and T4. Due to high (P<0.05) digestibility and assimilation of nutrients, significantly higher (P<0.05) DE(3.31kcal/g)was observed in T3, whereas lower DE (3.17 kcal/g) was in T1. However, T2 and T4 showed comparable findings for DE.

Haematological assay

Haematological parameters such as RBC, WBC, DLC (Lymphocytes, granulocytes and monocytes), PCV, Hb, MCV, MCH and MCHC were analysed from the blood of *P. hypophthalmus* supplemented with GP incorporated diets.

GP treated groups showed higher (P<0.05) RBC and thrombocytes than control on Day 45 of the treatment. Significantly higher (P<0.05) RBC of 2.16x $10^6 \mu L^{-1}$ and thrombocytes (6.05x $10^4 \mu L^{-1}$) recorded in T3 group (Table 3).Total RBC in the present study fall in the range of RBC estimated in *P. hypophthalmus* by Galagarza et al. (2017). Higher RBC in GP treated groups may be attributed by sulphur components of allicin play an important role as antioxidant and immune stimulants (Rahman, 2003) to ward off RBC damaging free radicals. In agreement with the present study many researchers observed significantly higher (P<0.05) RBC in *O. niloticus, Clarias gariepinus, L. calcerifer, X. helleri, Eurasian Perch* and *P. fluviatilis* species of fishes fed with either GP or garlic extract at the dose rate of 0.5 to 2.0% and 0.5%, respectively (Shalaby et al., 2006; Nwabueze, 2012; Talpur and Ikhwanuddin, 2012; Kalyankar et al., 2013; Setijaningsih et al., 2021; Zare et al., 2021).

Table 3. Blood cells count of *P. hypophthalmus* supplemented with garlic powder

Treatments	RBC x 10^6 µL	WBC x 10^4 /µL	Thrombocyte x 10^4 /µL	Lymphocytes x 10^4 /µL	Granulocytes x 10^4 /µL	Monocytes x 10^4 /µL
0% (T1)	1.70 ±0.07^c	3.54 ±0.11^a	4.96 ±0.31^b	2.35 ±0.10^a	1.14 ±0.02^a	0.049 ±0.007
1% garlic (T2)	1.93 ±0.06^b	3.30 ±0.06^{ab}	5.39 ±0.26^{ab}	2.05 ±0.06^b	1.05 ±0.03^b	0.026 ±0.007
2% garlic (T3)	2.16 ±0.09^a	3.04 ±0.11^b	6.05 ±0.14^a	1.93 ±0.12^b	1.0 ±0.03^b	0.032 ±0.011
3% garlic (T4)	1.92 ±0.041^b	3.12 ±0.058^b	5.53 ±0.2^{ab}	2.05 ±0.05^b	1.02 ±0.01^b	0.025 ±0.006
SEM	0.06	0.09	0.24	0.092	0.028	0.01
P Value	0.002	0.007	0.039	0.03	0.007	0.175
Test	**	**	*	*	**	NS

** -Significant at P<0.001 * - Significant at P<0.05 and NS – Non-significant. Means with different superscripts in a column differ significantly

In contrast to the present findings, Jahanjoo et al. (2018) observed significantly lower (P<0.05) RBC in the fry of Sobaity Sea Bream (*S. hasta*) supplemented with GP1%and found higher in the groups supplemented with the mixture of GP1%+ginger1%+thyme 1%. Whereas, Zaefarian et al. (2017) and Saleh et al. (2020) did not find any significant (P<0.05) effect of GP supplementation

on RBC of on brown trout (*S. caspius*) and *Dicentrarchuslabrax,* respectively.

Significant difference (P<0.05) in WBC count was observed (Table 3).Control group (T1) showed highest (P<0.05) count of WBC which was comparable with T2, while at 2 and 3 % of GP supplementation revealed decrease (P<0.05) in WBC count it may due to antimicrobial properties of active sulphur compound of garlic keeps the animal free from infection (Lee and Gao, 2012). However, total WBC count in the present study was fall in the range of WBC observed in *P. hypophthalmus* by Galagarza et al. (2017). Similar to present study Jahanjoo et al. (2018) and Nwabueze, (2012) who recorded significantly lower (P<0.05) WBC in the fry of Sobaity Sea Bream (*S. hasta*) and *C.gariepinus*,respectivelyon GP supplementation. In contrast to the present study, significantly higher WBC was recorded in other species supplemented with garlic such as *D.labrax* (Saleh et al.,2020), *Latescalcerifer* (Talpur and Ikhwanuddin, 2012), sword tail, *Xiphophorushelleri* (Kalyankar et al., 2013) hybrid tilapia, *O. niloticus x O.aureus* (Ndong and Fall, 2011) and in Eurasian Perch *P. fluviatilis* (Zare et al., 2021).Moreover, Aly and Mohamed (2010);Setijaningsih et al. (2021) and Zaefarian et al. (2017) did not find significant (P>0.05) effect on the WBC of *O. niloticus* and brown trout (*S. caspius*) respectively fed with garlic incorporated diets.

Significant differences were observed in lymphocytes and granulocytes (Table 3). Moreover, the pattern of significance was similar, where highest value (P<0.05) was observed for control (T1) while low and comparable for T2, T3 and T4. DLC observed in the presented study are in the range of leucocytes mentioned by Galagarza et al. (2017) in *P. hypophthalmus*. In accordance to the present study Aly and Mohamed (2010) and Jahanjoo et al. (2018) recorded significantly (P<0.05) decrease number of lymphocytes in *O. niloticus* and in the fry of *S. hasta*, respectively supplemented with GP supplementation. In contrast to the present findings, *L.calcerifer* (Talpur and Ikhwanuddin, 2012) supplemented with garlic (0.5-2%) showed higher (P<0.05) lymphocytes than control. Whereas, Zare et al. (2021) did not reveal significant difference (P>0.05) in lymphocytes (%) of Eurasian Perch *P. fluviatilis* supplemented with GP (1-3%).

Similar to that of granulocytes values observed in present study, Jahanjoo et al. (2018) recorded reduced count of (P<0.05) granulocytes in *S. hasta*fed with GP (1%) alone than that mixture of GP1%+ginger1%+thyme1% incorporated diets. Similarly, *L. Calcerifer* supplemented with garlic (0.5-2%) showed higher (P<0.05) granulocytes than control (Talpur and Ikhwanuddin, 2012).Results of the present study are in agreement with Zare et al. (2021) who observed non-significant difference (P>0.05) in phagocytic activities of *P. fluviatilis* under treatment of GP (1-3%) which may indicate antimicrobial activities of garlic. Moreover, Jahanjoo et al. (2018) also observed significantly lower (P<0.05) monocytes in *S. hasta* fed with GP1%.

Significantly higher (P<0.05) PCV of 31.4% and Hb (11.5 g/dL) were recorded in T3 treated group (Table 4).It might be due to antioxidant and immune stimulants properties of garlic towards RBC damaging free radicals and improves overall health of fish (Rahman, 2003).In accordance to present study, garlic supplementation revealed higher (P<0.05) PCV and Hbin various species of fishes, such as *O. niloticus* (Shalaby et al., 2006), sword tail *Xiphophorushelleri* (Kalyankar et al., 2013), *L. calcerifer* (Talpur and Ikhwanuddin, 2012). Similarly, Saleh et al. (2020) observed higher (P<0.05) Hb in *D. labrax* supplemented with garlic but did not find significant effect (P>0.05) on PCV in the same.

Table 4. Packed cells volume, Hemoglobin and red blood cells indices of *P. hypophthalmus* supplemented with garlic powder

Treatments	PCV (%)	Hb (g/dL)	MCV (fL)	MCH (pg/cell)	MCHC (g/dL)
0% garlic (T1)	27.0 ±0.894 ^{b}	9.50 ±0.548^{b}	159.1 ±2.49^{a}	55.7 ±1.55	35.1 ±1.17
1% garlic (T2)	29.2 ±0.80 ^{ab}	9.85 ±0.35^{b}	154.7 ±1.60^{a}	52.1 ±0.89	33.7 ±0.61
2% garlic (T3)	31.4 ±0.748^{a}	11.50 ±0.5^{a}	145.6 ±2.69^{b}	53.3 ±2.06	36.6 ±1.15
3% garlic (T4)	29.4 ±0.510^{a}	10.1 ±0.368^{b}	153.1 ±1.04^{a}	52.5 ±0.91	34.3 ±0.77
SEM	0.75	0.45	2.06	1.44	0.95
P Value	0.007	0.031	0.003	0.313	0.209
Test	**	*	**	NS	NS

** -Significant at P<0.001 * - Significant at P<0.05 and NS – Non-significant. Means with different superscripts in a column differ significantly. PCV – Packed cell volume, Hb- Haemoglobin, MCV -Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC- Mean corpuscular haemoglobin concentration, g/dL- gram per decilitre, fL- femtolitre, pg/cell- picogram per cell

MCV and MCH value estimated in the present study are in the range described by Galagarza et al. (2017) in *P. hypophthalmus*.Result of the present study is in agreement with Zaefarian et al. (2017) who observed non-significant difference in MCH of brown trout (*S. caspius*) supplemented with GP. In contrast to present findings,Shalaby et al. (2006) recorded higher (P<0.05) MCV in *O. niloticus* fed GP at 2%.

CONCLUSION

Inclusion of garlic powder revealed improved performance in terms of feed assimilation efficiency, nutrient digestibility, utilization, RBC and Hb levels. Inclusion of 2% garlic powder supplementation was found better among different levels used in present experiment.

ACKNOLEDGEMENT

The authors are grateful to Department of Animal Nutrition, Veterinary Pathology and Krushi Vigyan Kendra, Navsari for providing necessary technical support and infrastructure for conducting the experiment.

References

Adler, A.J. and Holub, B.J. 1997. Effect of garlic and fish-oil supplementation on serum lipid and lipoprotein concentrations in hypercholesterolemicmen. American Journal of Clinical Nutrition. 65(2): 445-450.

A. O. A. C. 2005. Official Methods of Analysis of Association of Official Analytical Chemists, 18th Edn, Maryland, USA.

Ahmed, N. and Hasan, M.R.2007.Growing pangas industry faces constraints in Bangladesh. Global Aquaculture Advocate.10: 60-62.

Ali, M.L., Haque, S.M., Wahab, M.A. and Borski, R.J. 2015. Development of Pangasius catfish culture technology in hyposaline waters. Aquanews.30(3): 1-4.

Ali, M. and Ibrahim, I. S. 2019. Phytochemical screening and proximate analysis of garlic (*Allium sativum*). Inorganic Chemistry. 4: 478-482.

Aly, S. M. and Mohamed, M. F. 2010. *Echinacea purpurea* & *Allium sativum* as immune stimulants in fish culture using Nile tilapia (*Oreochromis niloticus*). Journal of Animal Physiology and Animal Nutrition. 94(5): 31-39

Biswas, C., Hossain, M.M., Jaman, H.U., Roy, H.S., Alam, F., Bappa, S.B. and Khatun, A.2016. Dietary probiotics enhance the immunity of Thai pangas (*Pangasiushypophthalmus*) against *Pseudomonas fluorescens*. International Journal of Fish Aquatic Science. **4**(**4**): 173-178.

Blaxhall, P.C. and Daisley, K.W. 1973. Routine haematological methods for use with fish blood. Journal of Fish Biology. 5(6): 771-781.

Cullen, S.P., Monahan, F.J., Callan, J.J. and O'Doherty, J.V. 2005. The effect of dietary garlic and rosemary on grower-finisher pig performance and sensory characteristics of pork. Irish Journal of Agricultural. Food Research. 44: 57-67.

Dabrowski, K. 1986. Physiological and nutritional aspects. Aquaculture cyprinids, 55.

Da, C.T., Hung, L.T., Berg, H., Lindberg, J.E. and Lundh, T. 2013. Evaluation of potential feed sources, and technical and economic considerations of small scale commercial striped catfish (*Pangasius hypothalamus*) pond farming systems in the Mekong Delta of Vietnam. Aquaculture Research. 44(3): 427-438.

Dacie, J.V. and Lewis, S.N.1984. Practical Haematology. 6th Edn. Edinburg, Churchill Livingstone.

Darwish, A.M., Mitchell, A.J. and Straus, D.L.2009. Evaluation of potassium permanganate against an experimental subacute infection of *Flavobacteriumcolumnare* in channel catfish, *Ictaluruspunctatus* (Rafinesque). Journal of Fish Disease. 32(2): 193-199.

De la Noue, J. and Choubert, G. 1986. Digestibility in rainbow trout: comparison of the direct and indirect methods of measurement. The Prog. Fish-Cult. 48(3): 190-195.

Erguig M, Yahyaoui A, Fekhaoui M and Dakki M. 2015. The use of garlic in aquaculture. European Journal of Biotechnology and Bioscience.3(8):28-33.

Esmaeili, M., Abedian Kenari, A. and Rombenso, A.N.2017. Effects of fish meal replacement with meat and bone meal using garlic (*Allium sativum*) powder on growth, feeding, digestive enzymes and apparent digestibility of nutrients and fatty acids in juvenile rainbow trout (*Oncorhynchus mykiss,*Walbaum, 1792). Aquaculture Nutrition.23(6): 1225-1234.

FAO. 2020. Cultured Aquatic Species Information Programme. *Pangasiushypophthalmus.* Text by D. Griffiths, P. Van Khanh and T.Q. Trong. *In* FAO Fisheries and Aquaculture Department [online]. Rome. (Accessed 23 March 2020). Available: http://www.fao.org/fishery/culturedspecies/Pangasius_hypophthalmus/en.

Fletcher, R. 2020, October 11. Catfish can be key drivers of global aquaculture growth. The fish site blog. https://thefishsite.com/articles/catfish-can-be-key-drivers-of-global-aquaculture-growth

Galagarza, O.A., Kuhn, D.D., Smith, S.A. and Hrubec, T.C.2017. Hematologic and plasma chemistry RIs for cultured striped catfish (*Pangasiushypophthalmus*) in re circulating aquaculture systems. Veterinary Clinical Pathology. 46(3): 457-465.

Gilannejad, N., Silva, T., Rodriguez, G.M. and Yufera, M. 2019. Effect of feeding time and frequency on gut transit and feed digestibility in two fish species with different feeding behaviours, gilthead seabream and Senegalese sole. Aquaculture.513: 734438

Hien, T., Nguyen, P.T., Le Tu, T. and Glencross, B. 2010. Assessment of methods for the determination of digestibility of feed ingredients for Tra catfish, *Pangasinodon hypothalamus*. Aquacuture Nutrition.16: 351-358.

Huang, W., Yao, C., Liu, Y., Xu, N., Yin, Z., Xu, W., Miao, Y. Mal, K. and Ai, Q. 2020. Dietary allicin improved the survival and growth of large yellow croaker (*Larimichthy scrocea*) larvae via promoting intestinal development, alleviating inflammation and enhancing appetite. Frontiers in Physiology.11:587674.

Hung, L. T., Liem, P. T., Tu, H. T. and Mariojouls, C.2002. Comparing growth and protein requirements for fingerlings of three catfish of the Mekong River (*Pangasiusbocourti, Pangagasiushypothalmus and Pangasiusconchophilus*). Journal of Aquaculture in the Tropics. 17(4): 325-335.

Jahanjoo, V., Yahyavi, M., Akrami, R. and Bahri, A. H. 2018. Influence of adding garlic (*Allium sativum*), Ginger (*Zingiberofficinale*), thyme (*Thymus vulgaris*) and their combination on the growth performance, haemato immunological parameters and disease resistance to *Photobacterium damselae* in sobaity sea bream (*Sparidentex hasta*) Fry. Turkish Journal of Fisheries and Aquatic. Sciences. 18(4): 633-645.

Jegede, T. 2012. Effect of garlic (*Allium sativum*) on growth, nutrient utilization, resistance and survival of *Tilapia zillii* (Gervais 1852) fingerlings. Journal of Agriculture Sciences. 4(2): 269.

Jeyakumari, A., Ninan, G., Joshy, C.G., Parvathy, U., Zynudheen, A.A. and Lalitha, K.V. 2016. Effect of chitosan on shelf life of restructured fish products from pangasius (*Pangasianodonhypophthalmus*) surimi during chilled storage. Journal of Food Science and Technology.53(4): 2099-2107.

Kalyankar, A.D., Gupta, R.K., Bansal, N., Sabhlok, V.P. and Singh, D. 2013. Effect of garlic (*Allium Sativum*) against *AeromonasHydrophila* and health management of Swordtail, *Xiphophorus Helleri* A.D. Jornal of Environmental Science and Sustainability. 1(2): 41–48.

Kaur, H. And Ansal, M.D. 2020. Efficacy of garlic powder as a growth promoting feed additive for higher growth and improved flesh quality in an Indian major carp, *Labeorohita* (Ham.) fingerlings. Methods. 9(13): 14.

Khalil, A.M., Yasuda, M., Farid, A.S., Desouky, M.I., Mohi-Eldin, M.M., Haridy, M. and Horii, Y. 2015. Immuno modulatory and anti parasitic effects of garlic extract on Eimeriavermiformis-infected mice. Parasitology Research.114(7): 2735-2742.

Kim, S.R., Jung, Y.R., An, H.J., Kim, D.H., Jang, E.J., Choi, Y.J.and Chung, H.Y. 2013. Anti-wrinkle and anti-inflammatory effects of active garlic components and the inhibition of MMPs via NF-êBsignaling. PloS one. 8(9): e73877.https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0073877

Kumar, A., Harikrishna, V., Reddy, A.K., Chadha, N.K. and Rani A.M.B. 2017. Salinity tolerance of *Pangasianodon hypophthalmus* in inland saline water: effect on growth, survival and haematological parameters. Ecology Environment and Conservation.23 (1): 475-482.

Lee, J. Y. and Gao, Y. 2012. Review of the application of garlic, *Allium sativum*, in aquaculture. Journal of the World Aquaculture Society. 43: 447-458.

Lefevre, S., Huong, D.T.H., Nguyen, T.K., Wang, Nguyen, T.P. and Bayley, M. 2011. A telemetry study of swimming depth and oxygen level in a pangasius pond in the Mekong Delta. Aquaculture. 315(3-4): 410-413.

Lewis, W.H. and Elvin-Lewis, M.P.F. 2003. Medical Botany: Plants Affecting Human Health. Edn 2. New York, Wiley, 832

Naveenkumar, B.T., Kaur, Y., Tyagi, A.and Shanthanagouda, A.H. 2017. Baseline studies on selected hematological parameters of Indian major carps, exotic carps and catfishes. Journal of Fisheries and Life Sciences. 2: 60-67.

Ndong, D. and Fall, J. 2007. The effect of garlic (*Allium sativum*) on growth and immune responses of hybrid tilapia (*Oreochromis niloticus x Oreochromis aureus*).Journal of Clinical Immunology and Immunopathology Research. 3(1): 1-9

Nwabueze, A.A.2012. The effect of garlic (*Allium sativum*) on growth and haematological parameters of *Clariasgariepinus* (Burchell, 1822). Sustainable Agriculture Research.1(526-2016-37830).

Paulraj, R. And Easterson, D.C.V. 1982. Determination of digestibility coefficient. CMFRI special publication-Manual of Research Methods for Fish and Shellfish Nutrition. 8: 75-81.

Patel, P.P., Patel, M.P. and Tank, P.R. 2019. Effect of Herbal Liver Tonic on the Growth, Digestibility and Bio-chemical Composition of Grey Mullet *Mugilcephalus* (Linnaeus) fry. International Journal of Current Microbiology and Applied Sciences. 7(8): 2741-2749.

PrayogiSunu, D.S., Mahfudz, L.D. and Yunianto, V.D. 2019. Prebiotic activity of garlic (*Allium sativum*) extract on *Lactobacillus acidophilus*. Veterinary World. 12(12): 2046.

Rafiee, G.R., Saad, C.R., Kamarudin, M.S., Ismail, M.R. and Alavi, S.M.H. 2006. Estimation of ammonia excretion rates during a period of red tilapia, *Oreochromis* sp. Culture, considering biomass increase in a water recirculating system. Indian Journal Fisheries.6(1): 69-82.

Rahman, K. 2003. Garlic and aging: a new insight into an old remedy. Ageing Research Reviews. 2**:**39-56.

Rao, G.K. 2010. Present status of pangasius*, Pangasianodon hypophthalmus* (Sauvage) farming in Andhra Pradesh, India. A comprehensive study report, submitted to the National Fisheries Development Board (NFDB), Hyderabad, India, pp: 41

Saleh, N.E., Michael, F.R. and Toutou, M.M. 2020. Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcuslabrax*). Egyptian Journal of Aquatic Research. 41(2): 211-217.

Sawant, G.P., Singh, H., Sawant, N.H. and Yusufzai, S.I. 2003. Bioenergetic studies on the fry of *Liza parsia* (Hamilton- Buchanan, 1822) using three different oil cakes. Indian Journal of Applied and Pure Biology. 18(2):169-178.

Setijaningsih, L., Setiadi, E. and Taufik, I. 2021, April. The effect of garlic *Allium sativum* addition in feed to the growth performance and immune response of tilapia *Oreochromisniloticus*. In *IOP Conference Series:* Earth and Environmental Science. 744(1): 012072 I OP Publishing.

Shalaby, A.M., Khattab, Y.A. and Abdel Rahman, A.M. 2006. Effect of Garlic (*Allium sativum*) and Chloramphenicol on growth performance, physiological parameters and survival of Nile Tilapia (*Oreochromisniloticus*). The Journal of Venomous Animals and Toxins including Tropical Diseases. 12(2): 172-201.

Shrestha, M.K., Pandit, N.P., Bista, J.D. and Dahal, S.P. 2015. Polyculture of mixed-sex Nile tilapia (*Oreochromisniloticus*) with pangas catfish (*Pangasius hypophthalmus*). Nepalese Journal of Aquaculture and Fisheries .2: 86-93.

Silarudee, S., Tongpim, S., Charoensri, N. and Doolgindachbaporn, S. 2019. Effect of a Probiotic *Lactobacillus plantarum* CR1t5 dietary supplements on non-specific immunity in black eared catfish (*Pangasiuslarnaudii*). Journal of Pure Applied Microbiology. 13(1): 289-296.

Talpur, A.D. and Ikhwanuddin, M.H.D. 2012. Dietary effects of garlic (*Allium sativum*) on haemato-immunological parameters, survival, growth, and disease resistance against *Vibrio harveyi* infection in Asian sea bass, *Latescalcarifer* (Bloch). Aquaculture. 364: 6-12.

Tu-Tran, L.C., Nguyen, T.C., Verreth, J.A. and Schrama, J.W. 2020. Doses response of dietary viscosity on digestibility and faecal characteristics of striped catfish (*Pangasionodonhypophthalmus*). Aquaculture Research.51(2): 595-604.

Volstorf, J. 2021. *Pangasianodon hypophthalmus* (Findings). In: Fish Etho Base, ed. Fish Ethology and Welfare Group. World Wide Web electronic publication. Version 1. 327. www.fishethobase.net.

Wintrobe, M.M. 2008. *Wintrobe's clinical hematology* (Vol. 1). Lippincott Williams & Wilkins.

Xu, A., Shang Guan, J., Li, Z., Gao, Z., Huang, Y. and Chen, Q. 2020. Effects of garlic powder

on feeding attraction activity, growth and digestive enzyme activities of Japanese seabass, *Lateolabraxjaponicus*. Aquaculture Nutrition.26(2): 390-399.

Yigit, M., Koshio, S., Aral, O., Karaali, B.and Karayucel, S. 2003. Ammonia nitrogen excretion rate-An index for evaluating protein quality of three feed fishes for the black sea turbot. Israeli Journal Aquaculture.–Bamidgeh. 55(1): 69-76

Zaefarian, A., Yeganeh, S. and Adhami, B. 2017. Dietary effects of garlic powder (*Allium sativum*) on growth, blood indices, carcass composition, and lysozyme activity in brown trout (*Salmocaspius*) and resistance against *Yersinia ruckeri* infection. Aquaculture International. 25(6): 1987-1996.

Zare, M., Tran, H.Q., Prokesova, M. and Stejskal, V. 2021. Effects of Garlic *Allium sativum* powder on nutrient digestibility, haematology, and Immune and stress responses in Eurasian Perch *Percafluviatilis* Juveniles. Animals. 11(9): 2735.

Zewdie, A.K. 2019. The different methods of measuring feed digestibility: EC Nutrition. 14(1): 68-74.