



Effect of Fish Silage Supplemented Diets on Growth and Health Status of Pangas Catfish, *Pangasianodon hypophthalmus* Fry

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ABSTRACT

Background: Freshwater Asian catfish, *Pangasianodon hypophthalmus* (pangas) is one of the fastest growing exotic species throughout world including India. Most recently, it has been considered as a potential candidate species for carp diversification especially in northern states of India including Punjab and Haryana. In Pangas culture, fish meal is used as primary protein source pertaining to its excellent amino acid profile, palatability and high nutritive value. However, due to continuously rising cost, limited availability and quality variations of fish meal, there is need to find out less expensive alternative quality protein sources. Among these, fish silage is easy to prepare product with high nutritional value and shelf life. In this view, the experimental study was conducted to evaluate the possibility of replacing fish meal with fish silage supplemented diets on growth and health status of Pangas catfish.

Methods: Indoor study was conducted in FRP pools (1.5×1×0.75m) for 120 days (July – October 2018) to evaluate the effect of acid fermented fish silage (prepared from fish waste collected from local fish market of Ludhiana, Punjab) for Pangas catfish fry. To study the effect of fish silage, four experimental diets were formulated by replacing fishmeal @ 50% and 100% and mixture of soybean and groundnut meal @ 25% and 50% levels from control diet along with one reference diet without any animal protein source (neither fish meal nor fish silage).

Result: The overall results of present study revealed that fish silage can be incorporated (100% replacement of fish meal with fish silage) in the diet of Pangas catfish fry (*P. hypophthalmus*) with improved growth and feed utilization along with positive influence on health status of fish in terms of general haematology, biochemistry and anti-oxidant status.

Key words: Animal protein, Fish growth, Fish meal, Fish silage, *Pangasianodon*, Plant protein.

INTRODUCTION

Freshwater Asian catfish *Pangasianodon hypophthalmus* (commonly named as Pangas) belonging to family *Pangasidae*, is one of the fastest growing exotic species (native of Vietnam) in aquaculture. Over the last ten years, Pangas has emerged as a new aquaculture whitefish product in the world market (Singh and Lakra, 2012). In India, it was introduced in West Bengal from Bangladesh during 1997 and eventually to other states viz. Andhra Pradesh, Kerala, Odisha, where it is now well established and cultured under monoculture system, with a production range of 15-20 t/ha/yr (Singh and Lakra, 2012). In the recent past, pangas catfish has also been reared in northern states of Punjab and Haryana on experimental basis, as a potential candidate species for carp diversification, due to huge demand of fish having less intramuscular spines.

Fish Meal (FM) is being utilized as protein feed ingredient in fish feed including Pangas, due to its excellent amino acid profile, palatability and high nutritive value (Alceste and Jory, 2000). However, due to its rising cost, limited availability and uncertainties about its quality (New and Wijkstrom, 2002), there is need to find out less expensive alternative protein sources. A number of alternative protein resources such as meat and bone meal, hydrolyzed feather meal, flesh-meal and blood meal (Millamena, 2002) dried fish and chicken viscera (Giri *et al.*, 2000), poultry silage (Middleton *et al.*, 2001), crayfish meal

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(Agouz and Tonsy, 2003) and shrimp meal (Al-Azab, 2005) have been tried to replace FM either partially or fully, but these pooled meals of various animal sources are not sufficient to meet the growing demands of rising fish industry.

Fish processing generates solid wastes as high as 50-80% of the original raw material and if used properly, can be one of the excellent raw materials for the preparation of quality products, including protein feeds (Wassawa *et al.*, 2007). The three most common forms for utilization of fish processing waste include FM, fish oil and fish silage (FS).

FS is a fermented brown liquid product produced from the whole fish or parts of it, to which acids, enzymes or lactic-acid-producing microbes are added (FAO, 2003). Silage prepared by acid treatment is preferred over other methods due to its nutritional value and shelf life (Fagbenro and Jauncey, 1994). It is an attractive alternative to FM due to number of advantages such as simple and fast preparation technique, little investment, reduced effluents and odour problems. However, the major disadvantage is pasty voluminous product, implying an additional drying cost (Beerli *et al.*, 2004). Further, there are several reports regarding usage of FS as sole ingredient or as one of the ingredient in formulated feeds at different levels for different fish species (Haider *et al.*, 2016). With this background, the experimental study was conducted to evaluate the possibility of replacing traditional feed ingredients including FM with acid fermented FS supplemented diets on water quality parameters, survival, growth and health status (haematological, biochemical, lipid profile and antioxidant parameters) of Pangas (*P. hypophthalmus*) catfish.

MATERIALS AND METHODS

The experimental fish, Pangas were procured from West Bengal and acclimatized in cemented tanks at College of Fisheries, Guru Angad Dev Veterinary and Animal Sciences University (GADVASU), Ludhiana for one month (June 2018). For experimental study, twenty uniformly sized fishes (avg. Length 5-6 cm, avg. Weight 1-2 g) were stocked in FRP pools (1.5x1x0.75m) with continuous oxygen supply (in triplicate). 1-2 inch thick soil was spread at the bottom of FRP pools before filling of water. Borewell water was used for initial filling and exchange of water during the experimental period.

Feed composition, formulation and fish feeding

Five experimental supplemented pelleted diets were prepared by replacing FM @ 50 and 100% (D2 and D3) and a mixture of groundnut and soybean meal @ 25 and 50% (D4 and D5). One more experimental diet (D6) was prepared without any animal protein source (FM or FS) and D1 served as control (Table 1). For preparation of FS, fish waste (including viscera, scales, head, fins etc.) was procured from local fish market of Ludhiana. Waste was finely chopped and 4% formic acid (weight by volume) was added to lower the pH up to 3.5 along with butylated hydroxyl toluene (BHT) as antioxidant @ 250 mg/l. The mixture was stored at room temperature for a period of 30 days (30-35°C), with daily thorough mixing along with maintaining pH at 3.5 to avoid putrefaction (Oetterer, 2002). The silage was added to finely grounded ingredients as per feed formulation (Table 1) and floating feed pellets were prepared with extruder machine. The proximate composition of feed ingredients and experimental diets (Table 2) was analysed by following the methods of AOAC (1995). Floating pelleted feed was provided to the fish @ 5-3% fish body weight twice a day, for experimental period of 4 months *i.e.* July-October, 2018

(5% for 1st month, 4% for 2nd and 3rd month and 3% for 4th month). Amount of feed was adjusted at every monthly sampling according to increase in fish weight.

Water quality parameters

Water quality parameters in terms of water temperature, pH, dissolved oxygen (DO), total alkalinity (TA), ammonical nitrogen (NH₃-N), nitrate nitrogen (NO₃⁻-N), nitrite nitrogen (NO₂⁻-N) and orthophosphate (O-PO₄³⁻), were measured at fortnightly intervals by following the methods of APHA (2005).

Survival and growth parameters

Fish survival was estimated by counting fish at the end of experiment and comparing it with number of fish stocked. Fish growth in terms of total body length and body weight was recorded at monthly intervals. At the end of experiment, net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER) and condition factor (K) were calculated as follows:

NWG =

avg. final body weight - avg. initial body weight

SGR (% increase in weight/day) =

$$\frac{(\ln \text{ final body weight} - \ln \text{ initial body weight})}{\text{Culture days}} \times 100$$

ln = Natural Logarithm

FCR = Feed given (g) / Weight gain (g)

PER = Weight gain (g) / Protein intake (g)

K = Body weight / (Body length)³ × 100

Haematological parameters

Hematological parameters were analysed at the completion of experiment for haemoglobin (Hb) by method of Sahli (1962) and haematocrit (Ht) by micro-capillary method (Mukherjee, 1988).

Biochemical parameters and Lipid profile

Biochemical parameters, including total protein (TPROT) (Gornall *et al.*, 1949), albumin (ALB) and triglyceride (TRI) by method of Fossati *et al.* (1971), cholesterol (CHO) by modified Roeschlau *et al.* (1974) method. High density lipids (HDL), low density lipids (LDL), were analysed using bioanalytical test kits (Erba Diagnostic Mannheim GmbH kits) on a biochemical analyser (CHEM-7).

Antioxidant parameter

Blood hemolysate (RBC lysate) was used to analyse antioxidant parameters *i.e.* Superoxide dismutase (SOD) and lipid peroxidation (LPO) by following the methods of Nishikimi *et al.*, (1972) and Placer *et al.*, (1966) respectively at the completion of experiment.

Statistical analysis

Statistical analysis of the data was performed with a statistical package (SPSS 16.0 for Windows, SPSS Inc., Richmond, CA, USA). One way ANOVA was applied to work out the effect of FS supplemented diets on water quality parameters, survival, growth, haematological, biochemical and

antioxidant parameters of fish ($P \leq 0.05$). Significant ANOVA was followed by Duncan's multiple comparison to determine difference between treatments.

RESULTS AND DISCUSSION

Water quality

The results revealed that water quality parameters w.r.t water temperature (33.23 to 33.48°C); DO (10.61 to 10.96 mg l⁻¹); pH (7.07 to 7.15); TA (151 to 155 CaCO₃ mg l⁻¹); NH₃-N (0.05-0.06 mg l⁻¹), NO₃⁻-N (0.39 to 0.53 mg l⁻¹), NO₂⁻-N (0.04 to 0.06 mg l⁻¹) and O-PO₄³⁻ (0.05-0.07 mg l⁻¹) did not vary significantly (Table 3) among treatments and control and were well within the acceptable levels required for normal growth and physiological activities of fish. Consistency and

uniformity of water quality parameters among treatments revealed that the presence of acid in silage supplemented diets did not have any detrimental effect on water quality especially with respect to alkalinity and pH, which remained in optimum range for pangas catfish culture as described by Santhosh and Singh (2007) and Abedin *et al.* (2017).

Fish survival and growth

Survival (%) of fish was significantly higher ($p \leq 0.05$) in all the treatments as compared to control (93.33, 96.66 and 98.33% in D1, D2, D3; 100% in D4 and D5 and 96.66% in D6, respectively). Results revealed 100% fish survival in D4 and D5 *i.e.* diets having no FM, but having combination of FS and plant protein sources. According to Kamei *et al.* (2018) survival of pangas varied from 93.33 to 96.19%,

Table 1: Per cent composition of experimental diets (incorporation of fish silage by replacing plant/animal protein sources at different levels).

Ingredients	Experimental diets					
	Control (D1)*	D2	D3	D4	D5	D6
Rice bran	28	28	28	28	28	28
Groundnut meal	30	30	30	26.25	17.5	35
Soybean meal	30	30	30	26.25	17.5	35
Fish meal	10	5	-	-	-	-
Fish silage	-	5	10	17.5	35	-
Vitamin-mineral mixture	1.5	1.5	1.5	1.5	1.5	1.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5

*Standardized by College of Fisheries, GADVASU.

Table 2: Proximate composition (% DM basis) of different feed ingredients and experimental diets.

Ingredient/diet	Crude protein	Ether extract	Ash	Crude fiber	NFE
Rice bran	14.67	2.47	11.00	8.56	63.30
Groundnut meal	35.62	1.76	8.40	19.26	34.96
Soybean meal	41.67	2.18	4.53	6.77	44.85
Fishmeal	51.33	5.56	24.33	3.44	15.34
Fish silage	35.67	14.53	12.00	3.70	34.10
D1	32.43	2.43	9.39	10.54	45.21
D2	31.64	2.88	8.78	10.65	46.05
D3	30.87	3.32	8.16	10.78	46.87
D4	30.64	4.26	8.58	9.88	46.64
D5	30.11	6.47	9.54	8.23	45.65
D6	31.16	2.07	7.61	11.50	47.66

Table 3: Physico-chemical parameters of water in different treatments during the experimental period.

Parameters	Treatments					
	D1	D2	D3	D4	D5	D6
Temperature (°C)	33.48 ^a ±0.28	33.40 ^a ±0.26	33.30 ^a ±0.29	33.23 ^a ±0.29	33.26 ^a ±0.28	33.24 ^a ±0.29
pH	7.11 ^a ±0.08	7.07 ^a ±0.05	7.15 ^a ±0.08	7.11 ^a ±0.06	7.07 ^a ±0.09	7.07 ^a ±0.05
DO (mg l ⁻¹)	10.75 ^a ±0.19	10.66 ^a ±0.24	10.83 ^a ±0.21	10.71 ^a ±0.19	10.61 ^a ±0.16	10.96 ^a ±0.21
TA (CaCO ₃ mg l ⁻¹)	152 ^a ±2.53	154 ^a ±1.55	154 ^a ±1.81	155 ^a ±2.02	153 ^a ±2.40	151 ^a ±2.00
NH ₃ -N (mg l ⁻¹)	0.05 ^a ±0.007	0.05 ^a ±0.006	0.06 ^a ±0.007	0.05 ^a ±0.005	0.05 ^a ±0.004	0.05 ^a ±0.004
NO ₃ ⁻ -N (mg l ⁻¹)	0.53 ^a ±0.05	0.39 ^b ±0.04	0.48 ^{ab} ±0.05	0.47 ^{ab} ±0.04	0.45 ^{ab} ±0.04	0.45 ^{ab} ±0.04
NO ₂ ⁻ -N (mg l ⁻¹)	0.04 ^a ±0.02	0.06 ^a ±0.04	0.06 ^a ±0.02	0.06 ^a ±0.01	0.06 ^a ±0.03	0.06 ^a ±0.04
O-PO ₄ ³⁻ (mg l ⁻¹)	0.05 ^a ±0.002	0.06 ^a ±0.004	0.05 ^a ±0.001	0.07 ^a ±0.006	0.06 ^a ±0.003	0.05 ^a ±0.008

Values are Mean ± S.E., ($p \leq 0.05$), n=3.

Values with same superscript (a, b,.....d) in a row do not differ significantly ($p \leq 0.05$).

Table 4: Growth parameters of Pangas (*P. hypophthalmus*) in different treatments.

Parameters	Treatments					
	(Control) D1	D2	D3	D4	D5	D6
Av. initial length (cm)	6.06 ^a ±0.02	6.02 ^a ±0.04	6.04 ^a ±0.06	5.88 ^a ±0.04	6.00 ^a ±0.15	5.96 ^a ±0.04
Av. final length (cm)	10.27 ^b ±0.15	11.76 ^a ±0.30	11.40 ^{ab} ±0.10	11.39 ^{ab} ±0.63	12.03 ^a ±0.29	11.49 ^{ab} ±0.60
Av. initial weight (g)	1.97 ^a ±0.06	2.03 ^a ±0.06	1.94 ^a ±0.04	1.78 ^a ±0.03	1.85 ^a ±0.16	1.52 ^b ±0.04
Av. final weight (g)	15.10 ^{cd} ±0.40	18.00 ^b ±1.15	21.33 ^a ±0.66	17.33 ^{bc} ±0.66	17.90 ^b ±1.24	14.48 ^d ±0.58
NWG	12.67 ^{bc} ±0.33	15.33 ^{bc} ±1.45	19.00 ^a ±0.57	15.33 ^{bc} ±0.66	15.67 ^b ±1.20	12.33 ^c ±0.88
SGR	2.07 ^d ±0.03	2.27 ^{bc} ±0.08	2.47 ^a ±0.06	2.27 ^{bc} ±0.03	2.30 ^{ab} ±0.06	2.10 ^d ±0.06
PER	0.40 ^c ±0.03	0.50 ^b ±0.06	0.51 ^a ±0.03	0.43 ^b ±0.04	0.41 ^b ±0.06	0.40 ^c ±0.05
FCR	3.08 ^a ±0.55	2.58 ^a ±0.46	2.02 ^a ±0.23	2.12 ^a ±0.31	2.32 ^a ±0.46	2.29 ^a ±0.38
K	1.40 ^a ±0.04	1.12 ^a ±0.13	1.14 ^a ±0.08	1.20 ^a ±0.17	1.05 ^a ±0.15	1.00 ^a ±0.21
Survival (%)	93.33 ^c ±0.10	96.66 ^b ±0.25	98.33 ^{ab} ±0.15	100 ^a ±0.21	100 ^a ±0.53	96.66 ^b ±0.45

Values are Mean ± S.E., (p≤0.05), n=5.

Values with same superscript (a, b, d) in a row do not differ significantly (p≤0.05).

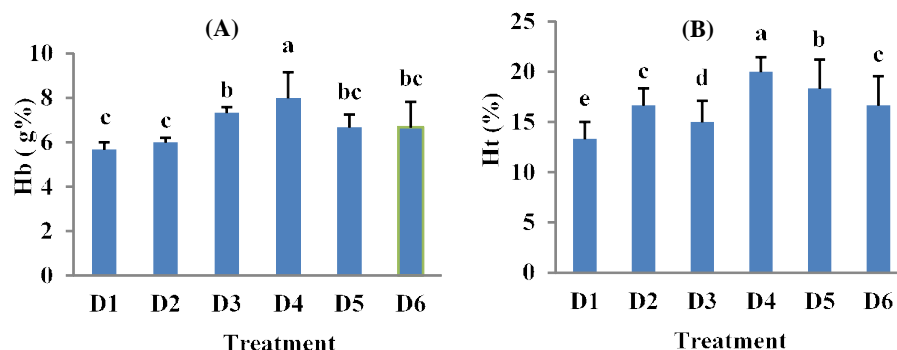


Fig 1: Changes in haematological parameters [A= Hb (g%), B= Haematocrit (%)] of fish in different treatments at the completion of the experiment.

when fed with diets, in which FM was replaced with blended protein source consisting of one third each of FS, groundnut oil cake and soybean meal @ 25%, 50%, 75% and 100% level. Maximum survival of 96.19% was observed in diet, where 75% of FM was replaced with blended protein source. Datta *et al.* (2018) reported poultry waste, FM, FS and soybean meal equally effective in terms of pangas survival (100% after 120 days of experiment).

Significantly higher ($p \leq 0.05$) fish growth in terms of FBW, NWG, SGR and PER was observed in D3 (21.33g, 19.00, 2.47 and 0.51). The differences for K and FCR were insignificant in all the treatments and control, however minimum value for FCR was observed in D3 (2.02) and maximum (3.08) in control D1 (Table 4). The findings of the present study are in line with results of previous study by Salah al-Din (1995), where inclusion of FS improved growth performance of catfish (*Clarias lazera*). Fagbenro and Jauncey (1994), also reported fermented fish-silage co-dried with protein feedstuffs as suitable protein supplement in case of juvenile catfish (*C. gariepinus*), which can provide up to 50% of dietary protein without affecting feed efficiency, fish growth or health. Better growth rate of fish fed with acid silage may be due to the presence of comparatively higher amount of free amino acids and active hydrolytic enzymes, released by the action of acid.

Soltan and Tharwat (2006) in their study found that

dried fish by-products silage can successfully replace up to 25 and 50% of fishmeal (FM) in tilapia and catfish diets without any negative effects on BW, WG and SGR, while the higher incorporation levels (50, 75 or 100%) significantly reduced the final BW for both the species. Fagbenro and Bello-Olusoji (1997) and Plascencia-Jatomea *et al.*, (2002) also revealed best FCR and PER in Nile tilapia fry fed with diets containing 0, 10 and 15% of shrimp head silage as a replacement to FM and higher FCR was obtained by the higher replacing levels (20, 25 and 30%). On the other hand, Wassef *et al.*, (2003) found that, partial or total replacement of FM by fermented FS alone or mixed with soybean meal did not significantly affected FCR and PER. Similarly, no significant differences in the weight gain, SGR, % survival and FCR were observed in *L. rohita* fingerlings by the dietary intake of acid ensilage @ 2.5, 5 and 10% (Tanuja *et al.*, 2017).

Haematological parameters

Significantly higher ($P \leq 0.05$) Hb (g %) and Ht (%) were recorded in all treatments as compared to control with maximum values of Hb and Ht (Fig 1) in D4 (8.00 and 20.00). Hb and Ht are important indicators of fish health. Hb is the protein contained in the red blood cells and is responsible for supplying oxygen to the tissue and for continuous supply of oxygen, sufficient Hb level should be maintained. An appropriate quantity and quality of protein is to be maintained

for keeping these parameters at a level required for optimized fish growth (Datta *et al.* 2018 and Khan *et al.* 2018). The results of the present study depict positive impact of FS supplementation on fish in terms of higher Hb and Ht may be due to its ability to stimulate blood formation (Habte-Tsion *et al.* 2013, Yones and Metwalli 2015). Further, according to Hedayati and Tarkhani (2014), 7.17 g % of Hb indicate good health of *P. hypophthalmus* and the values for Hb remained above 6.0 g % in all the treatments during the present study.

Biochemical parameters and lipid profile

Results obtained from the present study showed that the fish fed on experimental diets had significantly ($P \leq 0.05$) higher total protein, albumin and globulin (g dl^{-1}) as compared to control fed fish (D1) with highest values in D4 (4.32, 1.20 and 3.33) and lowest in D6 (2.47, 0.68 and 1.79). Alb/Glb ratio was significantly higher in D3 (0.58), however, the differences were insignificant among other treatments and control, except D5 (Fig 2). The results in terms of serum biochemical parameters improved in all silage treatments and remained at par or deteriorated as compared to control and are in line with hematological parameters showing maximum values in D4 (Fig 2). The increase in serum protein, albumin, globulin and Alb/glb ratio are considered to be associated with a strong immune response of fish (Wiegertjes *et al.*, 1996) coupled with improved haematological parameters resulting from enhanced nutritional status of fish. Similar observations were recorded

by Salah *et al.* (2014), who reported a significant ($P \leq 0.05$) increase in total protein and albumin levels in common carp, *Cyprinus carpio* where FM was replaced with fish bio silage at different levels (0, 25, 50 or 75%). The concentration of serum biochemical constituents (total protein, albumen, globulin, cholesterol, triglyceride, alkaline phosphatase, urea and creatinine concentration) did not vary significantly with dietary inclusion of acid ensiled fish waste silage @ 3,6 and 12% by replacing FM in Japanese quail (Tanuja *et al.*, 2018).

Lipid profile in terms of Cholesterol (mg dl^{-1}) and Triglycerides (mg dl^{-1}) showed an increase in all the experimental diets (Fig 3) as compared to control with highest values in D3 (366.53 and 56.00) and lowest in D1 (16.00) and D6 (193.20) respectively. Whereas, HDL (mg dl^{-1}) were found to be significantly higher in D1 (53.00) as compared to all the experimental diets with minimum values in D6 (10.30), whereas LDL and VLDL were found to be higher in all the treatments as compared to control with maximum values in D3 (110.00 and 11.32 mg dl^{-1}). Improved triglycerides and cholesterol can be co-related with better health status of fish resulting in improved survival and growth. Low concentration of plasma triglycerides usually corresponds to poor lipid reserves (Wagner and Congleton, 2004), while elevated triglycerides may be due to an imbalanced diet (Lemaire *et al.*, 1991). Goda *et al.*, (2007) too reported higher body lipid content for African catfish, *C. gariepinus*, fed with diets containing either poultry by-product meal (75%) or soybean meal (100%), as observed in present study. These results are also in agreement with the results

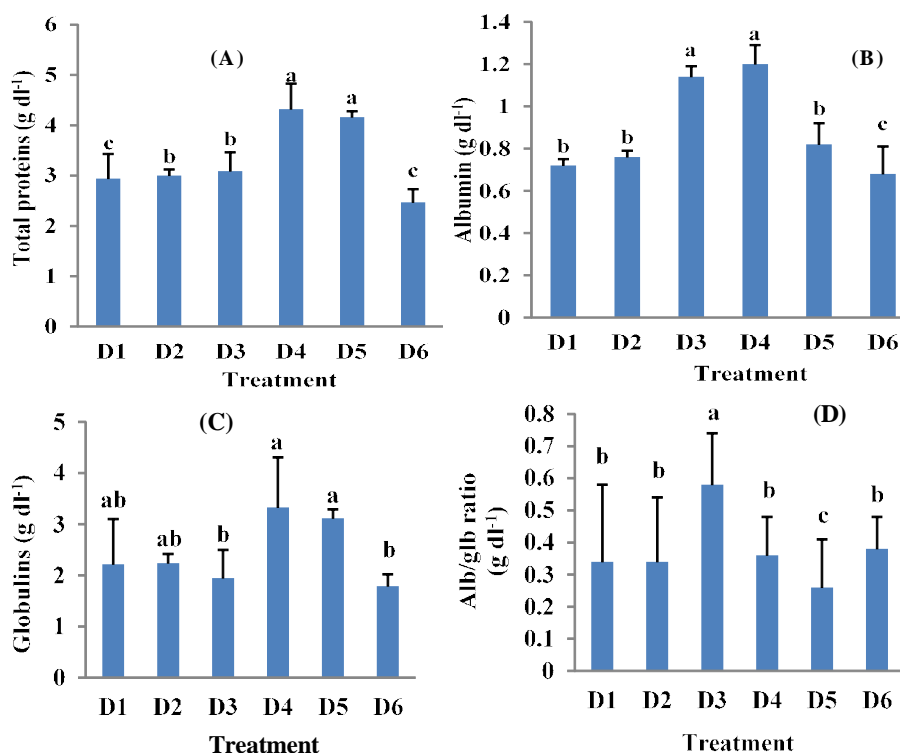


Fig 2: Changes in biochemical parameters [A=Total proteins (g dl^{-1}), B=Albumin (g dl^{-1}), C= Globulins (g dl^{-1}), D=Albumin/globulin ratio (g dl^{-1})] of fish in different treatments at the completion of the experiment.

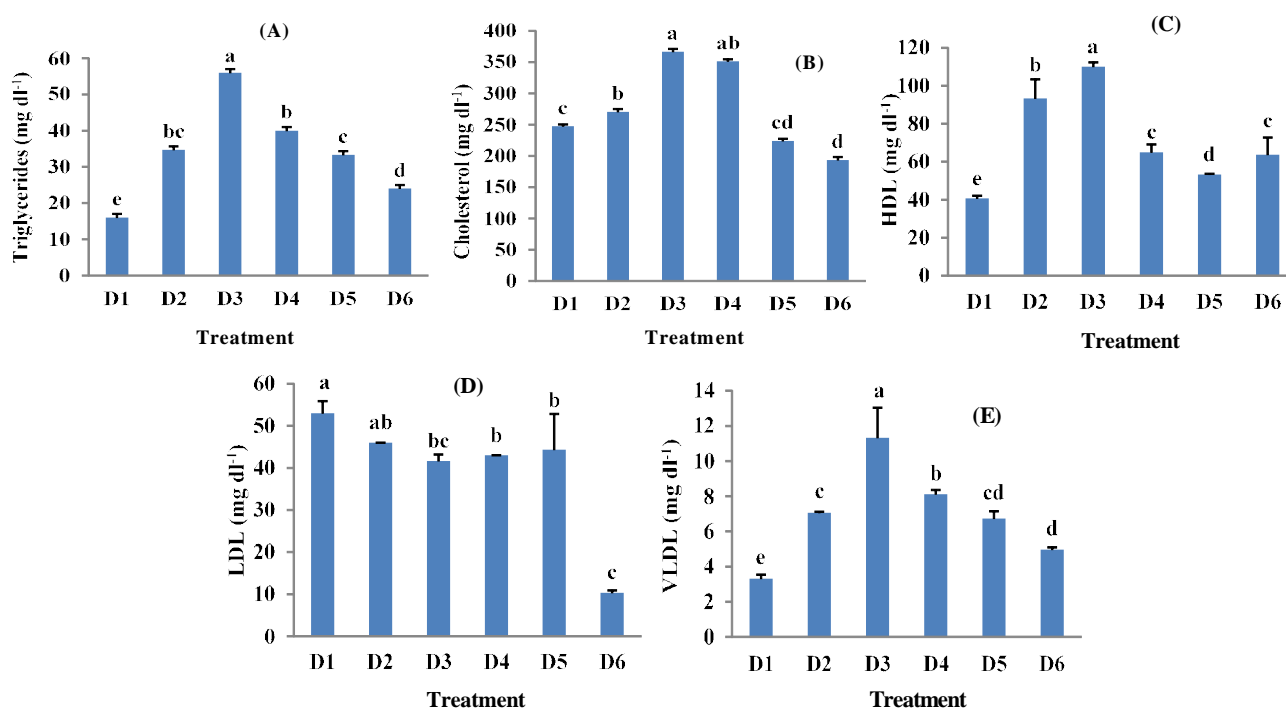


Fig 3: Changes in lipid profile [A= Triglycerides (mg dl⁻¹), B = Cholesterol (mg dl⁻¹), C= HDL (mg dl⁻¹), D= LDL (mg dl⁻¹), E= VLDL (mg dl⁻¹)] of fish in different treatments at the completion of the experiment

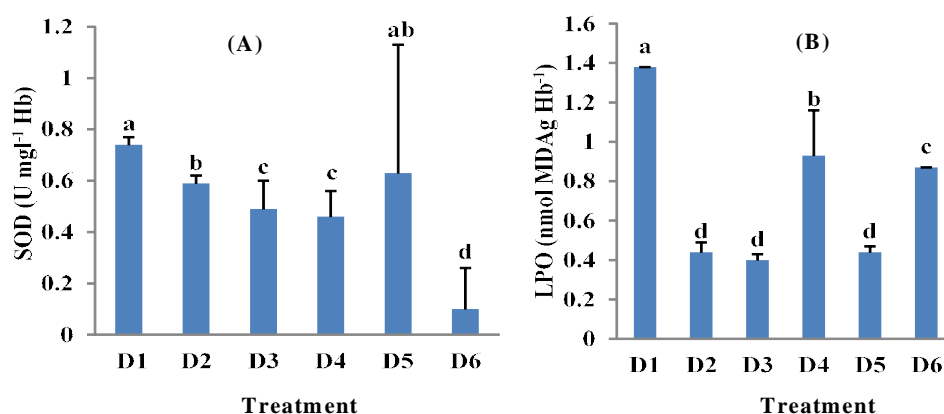


Fig 4: Changes in antioxidant parameters [A= SOD (U mg l⁻¹ Hb), B= LPO (nmol MDAg Hb⁻¹)] of fish in different treatments at the completion of the experiment.

of Gouveia (1992), who reported an increase of body lipid with the inclusion of poultry by-product in diets of rainbow trout, *O. mykiss*. Likewise, the serum triglyceride concentration increased significantly ($P \leq 0.05$) with 10% inclusion level of fish silage in broiler chicken (Boitai *et al.*, 2018).

Antioxidant parameters

SOD (U mg l⁻¹ Hb) and LPO (nmol MDAg Hb⁻¹) was found to be significantly higher ($P \leq 0.05$) in D1 (0.74 and 1.38), with minimum values of SOD in D6 (0.10) and LPO in D2 and D5 (0.44) (Fig 4). SOD and LPO are considered as indicators of immune status of any organism. The findings of the present study are in line with Lin and Lou (2011), who studied effects of inclusion of soybean meal at 0, 25, 50, 75 and 100% levels in replacement for FM in practical diets for

juvenile tilapia, *O. niloticus* × *O. aureus*. Results indicated that SOD activity decreased as compared to control, when the substitution level was more than 50%. Similar observations were recorded by Gui *et al.*, (2010) who reported a decreased antioxidant activity in crucian carp (*Carassius auratus gibelio*), when fed with different dietary levels (0, 10, 50 and 100g kg⁻¹) of cottonseed meal protein. Qi-you *et al.* (2008) too reported similar observations while studying effects of partial replacement of FM with soy protein isolated and meat bone meal (30%, 20% and 10%) on growth and non-specific immunity in rainbow trout (*O. mykiss*).

CONCLUSION

It can be concluded from the present experiment that FS can be incorporated (100% replacement of FM with FS) in

Pangas (*P. hypophthalmus*) feed to improve growth and feed utilization along with positive influence on health status of fish in terms of general haematology, biochemistry, and anti-oxidant status.

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