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***Research Article** (Open Access)*

EFFECT OF DIETARY SUPPLEMENTATION ON GROWTH, NUTRIENT UTILISATION AND DIGESTIVE ENZYMES IN ASIAN CATFISH, *PANGASIANODON HYPOPHTHALMUS* IN GROW OUT CULTURE SYSTEMS

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ABSTRACT
A 90 day feeding trial was undertaken to determine the dietary supplementation levels on Asian catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) fingerlings. Four practical (isonitrogenous and isoenergetic) dietary supplementation diets with 6% lipid level namely DS-1, DS-2, DS-3 and DS-4 were prepared. Each diet was assigned to triplicate group with a mean weight of 3.13±0.05 g and fed twice daily for 90 days. Weight gain % and specific growth rate (SGR) were highest (P< 0.05) in DS-2 (637.22±12.18 & 0.59±0.05, respectively), followed by DS-3 and DS-1 (30% CP diets) and lowest was in DS-4. Lowest FCR (1.35) and highest feed efficiency ratio (0.50) were observed in DS-2. Lowest protein efficiency ratio (2.11) and apparent net protein utilisation (ANPU) (19.23) was observed in DS-1 and DS-2, respectively. The highest survival rate (%) in the DS-2 group (99%) followed by other groups. The survival rate among the experimental groups did not vary significantly (P>0.05). Highest moisture content of fish was found insignificant among dietary treatments (P>0.05), whereas ash content and crude protein content of fish was highest in DS-4. Digestive enzymes like protease and lipase were higher in fishes fed with DS-3 while amylase was found lowest in the same group. ALT activity in muscle increased with dietary supplementation concentration in diet and afterwards decreases whereas, ALT activity in liver showed the opposite trend. The present study revealed that dietary supplementation at different concentrations was affected the growth and survival of pangasius and better utilisation of nutrients.

Keywords: Pangasius, Dietary supplements, Growth and Nutrient utilisation**.**

INTRODUCTION
Pangasianodon hypophthalmus is one of the candidate species for aquaculture because of its omnivorous feeding habits, rapid growth, disease resistance, and ease in the acceptance of artificial feeds. It was introduced into India during 1997 accidently via Bangladesh and there is a continuous eagerness among the fish farmers in the Andhra Pradesh for its culture and propagation. Pangasius can be cultured as monoculture or polyculture. Pangasius can grow to 1.5 to 3.0 kg in one year, and at an annual yield of 10 to 15 t/ha in pond culture. Pangasius culture was estimated about 40,000 hectares in the country with the current production levels of 500,000 to 600,000 metric tons per year (NFDB, 2008). It has good market in north and north-eastern states of India (Lakra and Singh, 2010). Nowadays, the expansion of culture of this species faces a major problem due to lack of a good quality of seed and lack of knowledge about the nutritional requirement of *P. hypophthalmus* in growout culture system.
Pangasius farming in India developing in the rapid mode, facing major challenges like lack of quality feed, ingredient shortage and decline in market price. Unavailability of good quality of feed is due to lack of information regarding the actual nutritional requirement of *P. hypophthalmus.* The feed which is formulated and used for *P. hypophthalmus* farming at present is based on the nutritional requirement of Tra catfish of Mekong delta. Hence, there is a need to emphasize on the optimum nutritional requirement

of *P. hypophthalmus,* which will provide the information about requirement of protein, lipid and carbohydrates and other nutrients.

Feed accounts 60% of total operational cost in a aquaculture enterprise. Low quality feed may lead loss of production due to nutritional deficiency may directly affect the productivity levels. Commercial feeds have been developed and evaluated as potential sources of nutrients for fish culture and also grow out farming of commercially important finfish and shell fish species. Digestion in animal metabolism determines the availability of nutrients needed for its biological functions. The study of the digestive physiology is a very important because nutrient efficiency depends on digestive process and functions of the digestive enzymes.

However, limited research work carried out on dietary supplementation of feeding aspects of *Pangasianodon hypophthalmus* using conventional feed ingredients which are locally available. As the fish is sold at lesser price in the international and domestic market due to its flesh quality which directly related to feed consumed by fish. Thus, the study required on effect of dietary supplementation on growth and survival of pangasius. Henceforth, the present investigation carried out on different dietary supplementation and their effect on growth, nutrient utilisation and survival of pangasius in growout culture system.

MATERIALS AND METHODS

***Experimental site and experimental animals*:** Experiment was conducted at MNR fish farm at Akiveedu, Andhra Pradesh. The fingerlings of pangasius (Average weight of 3.13 grams) procured from the farmers and reared in cultured tanks for 90 days. They were carefully transferred to another circular tank (1000 L) and were left undisturbed the whole night. The fishes were given a mild salt dip treatment (2.0%) for 2 min. The stock was acclimatized under aerated conditions with a formulated diet containing 35% crude protein for a period of 15 days.

Experimental design and set-up

The experiment was conducted as four treatments in triplicate in rectangular glass tanks at capacity of 160 L. The tanks were cleaned with potassium permanganate before the fishes stocked for the experimental trails. Completely Randomized Design (CRD) followed to conduct all the experimental trails. One hundred eighty fingerlings were randomly distributed in the six distinct experimental groups with three replicates each. Ten fishes with initial weight ranging from 3.13 g to 3.18 g were stocked in each plastic tub filled with 140 L chlorine free bore well water. The total volume of the water in each tub was maintained at 140 L throughout the experimental period. Round the clock aeration was provided. The aeration pipe in each tub was provided with an air stone and a plastic regulator to control the air pressure uniformly in all the tubs.

Formulation and preparation of experimental diets

Four diets with uniform protein, lipid and energy levels were prepared with a constant protein level of 40% and lipid content of 6% as dietary supplements. Ingredient composition was mentioned in the Table 1. Soya bean meal, ground nut oil cake along with feed attractant were treated as main dietary supplement in the experiments. The prepared diets were labelled as DS-1, DS-2, DS-3 and DS-4.Fish oil and sunflower oil used as lipid source. Each ingredient was dried and powdered before preparation of feed. Individual ingredients were weighed appropriately and mixed for each diet. Water was added to get dough of the mixture and then cooked it for 15 minutes. After that oil with 0.5% BHT and vitamin mineral mix were added. Pellets were prepared by pelletizer using a 3 mm dia. The pellets were oven dried and kept for 3-4 hr at 60^0C temperature. After drying, the pellets were packed in airtight container and labelled accordingly.

Table 1. Composition of the experimental diets (%).

Ingredient composition	Dietary supplement			
	DS-1	DS-2	DS-3	DS-4
Fish meal	22	24	26	28
Soyabean meal	28.25	26.25	24.25	22.25
GNOC	35.8	36.8	37.8	38.8
Ricebran	5.2	4.2	3.2	2.2
Wheat flour	4.2	4.2	4.2	4.2
Dietary supplement	0.5	1.5	2.0	2.5
Fish oil	3.0	3.0	3.0	3.0
Sunflower oil	3.0	3.0	3.0	3.0
Vitamin-mineral mix[2]	2	2	2	2
Feed attractant	0.5	0.5	0.5	0.5
Dry matter	94.76	94.37	93.71	95.27
Crude protein	40.02	40.49	40.98	40.66
Ether extract	6.34	6.30	6.53	6.05
Ash content	9.98	10.67	11.01	9.04
Nitrogen free extract	48.42	41.91	37.19	34.52

Composition of vitamin mineral mix

Vitamin A, 55,00,000 IU; Vitamin D3, 11,00,000 IU; Vitamin B2, 2,000 mg; Vitamin E, 750 mg; Vitamin K, 1,000 mg; Vitamin B6, 1,000 mg; Vitamin B12, 6mcg; Calcium Pantothenate, 2,500 mg; Nicotinamide, 10 g; Choline Chloride,150 g; Mn, 27,000 mg; I, 1,000 mg; Fe, 7,500 mg; Zn, 5,000 mg; Cu, 2,000 mg; Co, 450 L-Lysine, 10 g; DL-Methionine, 10 g; Selenium, 50 ppm; Satwari, 2500 mg.

Proximate composition analysis

The proximate composition of the experimental diets and fish muscle was determined following the standard methods of AOAC (1995) (Table 1). The moisture content was determined by drying at 105^0C to a constant weight. Nitrogen content was estimated by automated Kjeldahl apparatus (2200 Kjeltec Auto distillation, Foss Tecator, Sweden) and Crude protein (CP) was estimated by multiplying nitrogen percentage by 6.25. Ether extract (EE) was measured using a Soxtec system (1045 Soxtec extraction unit, Tecator, Sweden) using diethyl ether (boiling point, 40-60^0C) as a solvent and ash content was determined by incinerating the samples in a muffle furnace at 600^0C for 6 h. Total carbohydrate was calculated thus: total carbohydrate % = 100-CP% + EE% + Ash%).

Growth parameters

Growth and feed efficiency parameters were calculated based on the following formulae:
Survival (%) = 100 x FN/IN (IN, FN = Initial and Final Number of fish respectively)
Specific growth rate (SGR) % = [ln FW− ln IW]/number of culture days x100
Food conversion ratio (FCR) = dry feed intake (g) /wet weight gain (g)
Feed efficiency ratio (FER) = Net weight gain (g)/Body weight gain (g)
Protein efficiency ratio (PER) = (final biomass – initial biomass)/(total feed intake x dietary protein).
Apparent Net Protein Utilisation (ANPU) = Final body protein-Initial body protein/protein fedx100

Enzyme assays

In each treatment six fishes were used for the enzyme analysis and their intestine were dissected and frozen immediately at -20^0C for enzyme assays. Whole intestine was homogenized in cold 0.25 M sucrose in a Teflon coated motor driven homogenizer so as to prepare a 5% homogenate. The homogenate was centrifuged at 5000 g for 15 min in a cooling centrifuge (5^0C), the supernatant collected, frozen in sample vials and stored at -20^0C until assayed for the digestive enzymes.

Proteases

Proteolytic enzyme activity was determined by the casein digestion method of Kunitz (1947). The reaction mixture consisted of 1% casein as substrate, phosphate buffer (pH 7.5) and tissue homogenate which was incubated at 37^0C for 20 min. The reaction was stopped by adding 5% TCA and kept for 1 h at 2^0C. Samples were filtered and OD of supernatant was measured at 280 nm. A reagent blank was prepared by adding tissue homogenate just before stopping the reaction with no incubation. The activity of proteolytic enzyme was determined from the tyrosine standard curve and expressed as mole of tyrosine released/min/mg protein at 37^0C.

Lipase

Lipase activity was determined based on Cherry and Crandell (1932). The quantity of fatty acid released in unit time measured by the quantity of NaOH required to maintain constant pH. The reaction mixture consisted of distilled water, tissue homogenate, phosphate buffer solution (pH 7) and olive oil emulsion. The mixture was shaken well and incubated at 4^0C for 24 h. 95% alcohol and 2 drops of phenolphthalein indicator were added in the mixture and titrated against 0.05 N NaOH until the appearance of permanent pink color. A control was taken using an enzyme source that was inactivated prior to addition of buffer and olive oil emulsion. The milliequivalent of alkali consumed was taken as a measure of the activity of the enzyme.

Amylase

Amylase activity was assayed with 2% (w/v) starch solution as substrate (Rick and Stegbauer 1974). The 2% starch solution was prepared in phosphate buffer (pH 7). The reaction mixture was incubated at 37^0C for 30 min. Dinitrosalicylic acid (DNS) was added to stop the reaction and the mixture was kept in boiling water bath for 5 min. After cooling, the reaction mixture was diluted with distilled water and absorbance was recorded at 540 nm. Activity was determined from the maltose standard curve and expressed as mole of maltose released from starch/min/mg protein at 37^0C.

Alkaline phosphatase and acid phosphatase

Alkaline phosphatase (ALP) activity was determined by the method of Garen and Levinthal (1960). The assay mixture consisted of bicarbonate buffer (0.2M, pH 9.5), 0.1M $MgCl_2$, tissue homogenate and freshly prepared 0.1M para-nitrophenyl phosphate (p-NPP) as substrate. The reaction mixture was incubated in a water bath at 37^0C for 15 min and then stopped with 1ml of 0.1 N NaOH. OD was recorded at 410 nm. ALP activity was expressed as nanomoles p-nitrophenol released/min/mg protein at 37 ^{0}C. Acid phosphatase (ACP) activity was estimated using the same method as ALP, except that acetate buffer (0.2M, pH 5) was used in place of bicarbonate buffer.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) and the significant difference between the treatments was determined by Duncan's Multiple Range Test (DMRT) using SPSS (Version 16.0). Results are reported as mean ± SEM and each fish was considered as an experimental unit. The level of significance employed was $P<0.05$.

RESULTS AND DISCUSSION

Proximate composition of fish

Moisture content recorded on average 93.30% which was insignificant ($P>0.05$) among all the treatments. Crude protein deposition found significantly higher ($P<0.05$) in DS-3 and DS-4 group diet. Similarly, lower ($P<0.05$) fat deposition in fish muscle was observed in the DS-2 and higher in all other treatment groups which was insignificant ($P>0.05$). Ash content found highest in DS-4 and lowest in DS-3 significantly different among the dietary treatments.

Khan *et al*., (1993) reported that the whole body protein of *M. nemerus* increased significantly with increasing dietary protein level up to 42% and then decreased when fish fed higher protein levels. Similar results were found in other fishes like plaice, *Pleuronectes platessa* (Cowey and Pope 1972); juvenile tilapia, *Tilapia mosambicus* (Jauncey, 1982); silver barb, *Puntius gonionotus* (Wee and Ngamsane, 1987);

snakehead, *Channa micropeltes* (Wee and Tacon, 1982); golden mahseer, *Tor putitora* (Hossain *et al.*, 2002); climbing perch, *Anabas testudineus* (Hossain *et al.*, 2012); carps (Siddiqui *et al.*, 1988).

Table 2. Proximate composition of the fish tissue (% dry matter basis).

Treatments	Moisture	Organic Matter	Crude Protein	Ether extract	Ash	Crude fibre	Total carbohydrate
DS-1	6.83± 0.04[a]	93.17± 0.04[a]	18.99± 0.45[a]	8.14± 0.01[a]	4.64± 0.04[b]	1.88± 0.01[a]	58.53± 0.21[d]
DS-2	6.75± 0.03[a]	93.25± 0.03[a]	19.62± 0.62[b]	7.03± 0.08[b]	4.28± 0.03[b]	2.39± 0.02[c]	52.92± 0.70[c]
DS-3	6.54± 0.03[a]	93.46± 0.03[a]	20.41± 0.33[c]	8.15± 0.05[a]	3.25± 0.03[a]	2.08± 0.09[b]	47.80± 0.60[b]
DS-4	6.13± 0.03[b]	93.87± 0.03[a]	21.98± 0.65[d]	8.15± 0.06[a]	6.38± 0.03[c]	3.82± 0.06[d]	44.86± 0.69[a]

Mean values in the same column with different superscript differ significantly (P<0.05). Data expressed as Mean ± SE (n=3).

Growth parameters

Weight gain and Specific growth rate

In present study, highest weight gain was observed in DS-2 group (637.22±12.18) which was significantly different (P<0.05) from other groups and followed by other groups and minimum weight gain % was observed in control group. Weight gain percentage of *P. hypophthalmus,* juveniles increased with diet DS-2 group and decreased marginally subsequently when a further increase in dietary supplement concentration (Table 3). Feeding excess protein causes reduced growth rate because of the insufficient non-protein energy content in a high protein diet as part of the dietary protein metabolized and used for energy. Phillips (1979) suggested that increase in weight gain/ growth rate up to a certain protein level and decreasing thereafter may be consequence of an increase in the requirement of energy to get rid of excess toxic nitrogenous wastes because of an increase in the rate of amino acid breakdown (due to excess protein in the diet). Similar results were also found in studies carried out in other fishes like in silver barb, *Puntius gonionotus* fingerlings (Mohanta *et al.*, 2008).

Maximum SGR was found in DS-2 group (0.59± 0.06) which was significantly different (P<0.05) from other groups followed by DS-3 and DS-4 group and lowest SGR was found in the DS-1 group (0.51 ± 0.02). Specific growth rate of *P.hypophthalmus,* juveniles increased with the dietary diet fed up to DS-2 and decreased beyond it, with the increase in dietary protein level. It has been reported that the decrease in growth shown the optimum may be due to the reduction in dietary energy available for growth as extra energy is required to deaminate and excrete the excess amino acids absorbed (Jauncey *et al.*, 1982). Similar outcome were also found in studies carried out in other fishes like in bagrid catfish, *Pseudobagrus fulvidraco* (Kim and Lee, 2005); black catfish, *Rhamdia quelen*, fingerlings (Meyer and Fracalossi, 2004; Salhi *et al.*, 2004); which supported the present results.

Feed conversion ratio and feed efficiency ratio

Maximum value of FCR was found in DS-1 (1.60±0.01) which was significantly different (P<0.05) from other groups and minimum in DS-4 group (1.58±0.08). Fishes consume feed to satisfy nutrient and energy demands. It is believed that fish adjust food consumption to satisfy the metabolic energy requirements and once the minimum requirements are satisfied, the daily consumption may be related to dietary quality (De Silva and Anderson, 1995). In such cases, fish can adjust ingesting food as a function of DE contents in the diet. In contrast, a diet containing excess energy reduces feed consumption and thus lowers the intake of necessary amount of protein and other essential nutrients for maximum (NRC, 1993).

FER was found highest in DS-2 group (0.50±0.04) which was significantly different (P<0.05) from the other groups and minimum in DS-4 group (0.33±0.03). Similar results were also found in studies carried

out in different fishes such as in juvenile cobia, *Rachycentron canadum* (Chou *et al.*,2001); bagrid catfish, *Pseudobagrus fulvidraco* (Kim and Lee, 2005) which supported the present results.

Protein efficiency ratio

Maximum value of protein efficiency (PER) was found in DS-4 group (2.18±0.06) which was significantly different from the other groups and minimum in DS-1 group (2.11±0.08). PER was negatively correlated with dietary protein levels and consequently the efficiency of the dietary protein for biomass gain was lower in fish feed with high protein levels. The PER decreased significantly with the increase in dietary protein levels. Decrease in the PER with increased protein level indicate that maximum utilisation of protein for synthesis of body tissues occurs in low protein diets. It appears that the non-protein energy components have been maximally utilised as a source of energy, thus sparing protein for tissue synthesis in the low protein level diets. The present results are in agreement with those of Andrews (1977) who reported that feeding higher protein showed better growth in channel catfish fingerlings, whereas the lower protein level exhibited better protein conversion. A negative relationship between dietary protein levels and PER was observed in pacu (Bechara *et al.*, 2005). Apparently, this response is associated with the lack of digestible energy coming from non-protein sources in the diet with high levels of crude protein. The reason for the negative relationship between dietary levels of protein and PER was that a greater amount of protein in the diets was used as an energy source. When fishes are fed with these high protein diets, protein is then partially used as source of energy, diminishing its possibilities of use for deposition in tissues (Bicudo *et al.*, 2010).

Apparent net protein utilisation

In the present study, maximum value of Apparent Net Protein Utilisation (ANPU) was found in DS-1 group (22.41±0.32) which was significantly different ($P<0.05$) from the other groups and minimum in DS-2 group i.e. (19.13±0.50). It indicates that excess protein is catabolized to provide energy for growth (Adron *et al.*, 1976; Lied and Braaten, 1984.) Gurure *et al.*, (1995) observed a similar trend in Arctic char fed diets containing digestible protein levels from 23% to 55%. Steffens (1981) reported that an increase in dietary protein improves growth rate and feed conversion, but reduces PER and protein productive values in *Cyprinus carpio*. Similar results was also obtained in several studies in juvenile hybrid sturgeon, *Acipenser baerii* ♀× *A. gueldenstaedtii* ♂ (Guo *et al.*, 2012*)*; juvenile haddock, *Melanogrammus aeglefinus* (Kim and Lall, 2001); juvenile silver perch, *Bidyanus bidyanus* (Yang *et al.*, 2002).

Table 3. Growth parameters and nutrient utilization of different experimental groups fed different experimental diets.

Treatment	Weight gain %	SGR %	FCR	FER	PER	ANPU %
DS-1	588.37^{a} ± 10.48	0.51^{a} ± 0.02	1.60^{c}± 0.01	0.42^{a}± 0.003	2.11^{e}± 0.06	22.41^{f}± 0.32
DS-2	637.22^{ab} ±12.18	0.59^{ab}± 0.05	1.38^{b}± 0.09	0.50^{ab}± 0.03	2.13^{de}± 0.17	19.13^{e}± 0.50
DS-3	619.55^{bc} ±13.16	0.53^{bc}± 0.04	1.42^{a}± 0.04	0.30^{bc}± 0.02	2.16^{cd}± 0.08	20.47^{d}± 0.44
DS-4	523.96^{c}± 21.13	0.50^{c}± 0.06	1.58^{a}± 0.08	0.33^{c}± 0.04	2.18^{bc}± 0.10	21.78^{c}± 0.47

Data expressed as Mean ± SE n=3; Mean values in the same column with different superscript differ significantly (P<0.05).

Enzyme parameters

Protease

Proteolytic enzymes (or protease) are digestive enzymes which hydrolyze the peptide bond between the adjacent amino acid in proteins. The protease activity was found to be higher in intestine in the DS-3

group followed by DS-2 group and lowest activity was recorded in intestine in DS-1 group mentioned in Figure 1. There was an increase in protease activity in intestine with an increase in dietary supplements in the diet and further decrease in protease activity with increased level of dietary supplements. In contrast to this, trypsin was found to be unresponsive to dietary crude protein in early-weaned sea bass (Cahu and Infante, 1994). Trypsin and chymotrypsin activities were unresponsive to diet composition in Pintado (Lundstedt *et al.*, 2004). Lopez-Lopez *et al.*, (2005) also could not find any correlation between protease activity and dietary protein and between protease activity and growth in *Homarus americanus*.

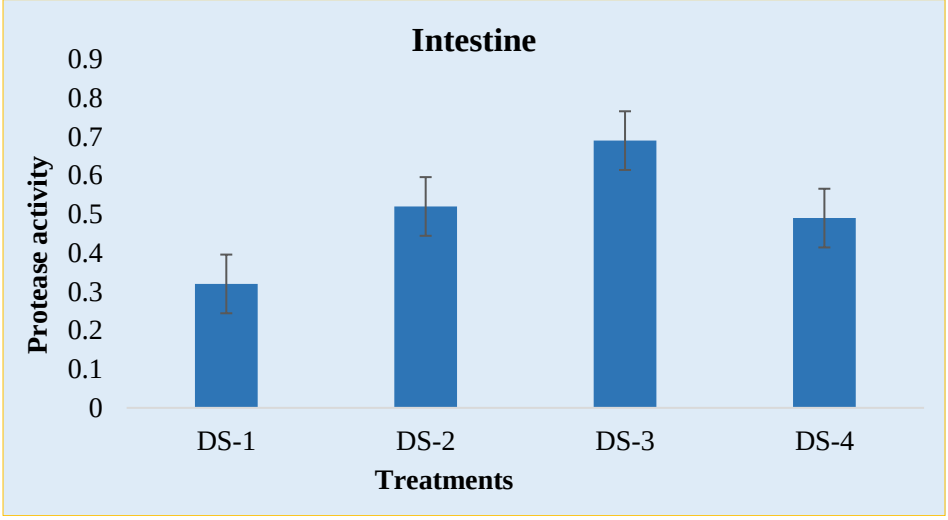

Figure 1. Protease activity of intestine of the different experimental groups (protease as micromol of tyrosine released/min/mg protein).

Lipase

In the intestine, the lipase activity was found to be higher in the DS-3 group followed by DS-2 and lowest activity in DS-1 and DS-4 group presented in Figure 2. Intestinal lipase activities in pangasius juveniles increased with dietary supplementation and decreased when further increase in dietary concentration.

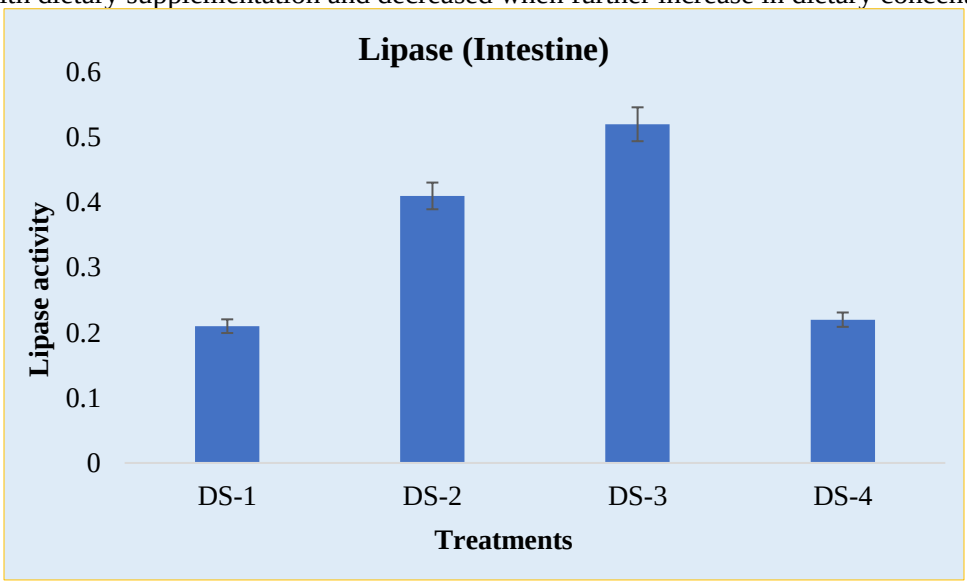

Figure 2. Lipase activity of intestine of the different experimental groups (lipase as units/mg protein).

Amylase

The intestinal amylase activity was found to be higher in the DS-2 group followed by DS-1 group and lowest activity was recorded in DS-4 group presented in Figure 3. Intestinal amylase activity in pangasius juveniles decreased with dietary supplementation levels increased in the diet. Low amylase activity in carnivorous fish (with stomach) and high activity in omnivorous fish (without stomach) is the general assumption (Sabapathi and Teo, 1993; Hidalgo *et al.*, 1999).

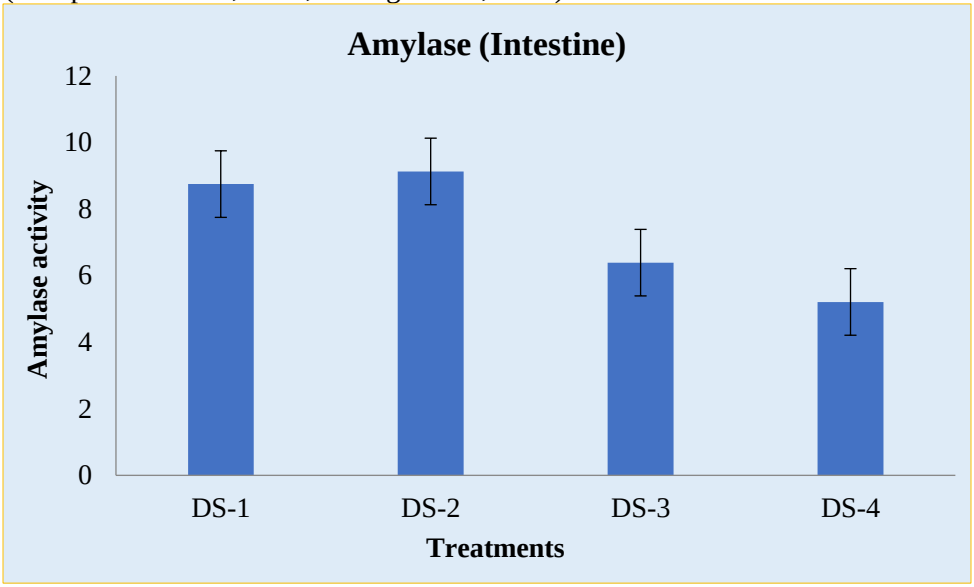

Figure 3. Amylase activity of intestine of the different experimental groups (amylase as micromol of maltose released/min/mg protein).

Alanine amino transferase (ALT) and aspartate amino transferase (AST)

ALT (Specific activities expressed as Nano moles of sodium pyruvate formed/mg protein/minute at 37^0C) and AST (Specific activities expressed as Nano moles of oxaloacetate released/min/mg protein at 37^0C) activities are usually used as an indicator of proper liver function in vertebrates and high AST and ALT values indicate the weakening or damage of normal liver function. In muscle, the highest activity of AST was observed in DS-1 group (52.49±1.17) which was significantly different from all other groups and the lowest activity was recorded in DS-4 group (30.71±1.12) mentioned in Figure 4. In the liver, the highest activity of AST was found in the group DS-3 (20.26±1.58) and the lowest activity has been recorded in DS-1 group (14.40±1.62) mentioned in Figure 5. In the present study, the AST activity in liver was found to be highest in DS-3 group, whereas in muscle activity was the lowest in DS-4 group, indicating negative control of excess protein on this enzyme. Decreased AST activity in muscle may suggest increase in the efficiency of the enzyme to synthesize non-essential amino acids for building a new protein because at lower protein supply in diet may lead to increase in catabolism of tissue protein. This suggests that muscle tissue is very efficient in utilizing amino acid for metabolic purposes. In both the liver and muscle tissue, AST predominates over ALT where the formation of amino acids via energy cycle is more through glutamate aspartate pathway. This represents the aerobic tendency of the tissues and glucose utilization for energy production. High protein intake enhances gluconeogenesis and amino acid catabolism (Cowey *et al.*, 1977). Alanine aminotransferase activity in liver and muscle showed opposite trend to that of AST in both the tissues. It seems there is complementary activity of these two amino acid metabolizing enzymes in a particular tissue (liver or muscle). Stress hormone cortisol induces gluconeogenesis, which mobilize the protein source and lipid source for glucose synthesis.

In the muscle, the highest activity of ALT was found in DS-4 group (33.53±1.76) and lowest activity was recorded in DS-1 group (27.22±1.67) which was significantly different from other groups mentioned in

Figure 6. In the liver, significantly higher value of ALT was recorded in DS-1 (12.83±0.71) and the lowest value is encountered in DS-4 group (5.27±0.36) presented in Figure 7. Similar observations were reported in *Cyprinus carpio* (Kheyyali *et al.*, 1989) and *Labeorohita* (Dalal *et al.*, 2001).

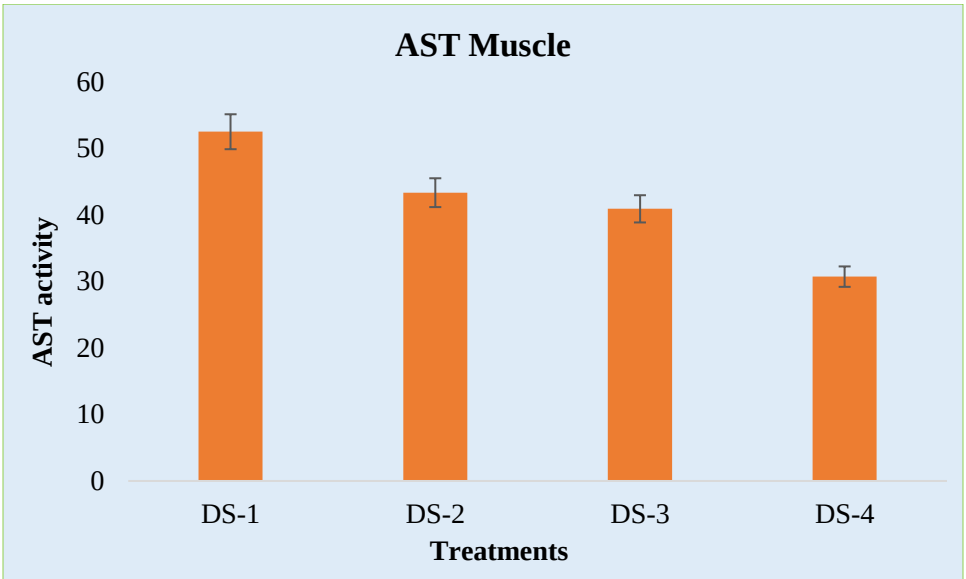

Figure 4. Aspartate amino transaminase (AST) activity of muscle in the different treatment groups.

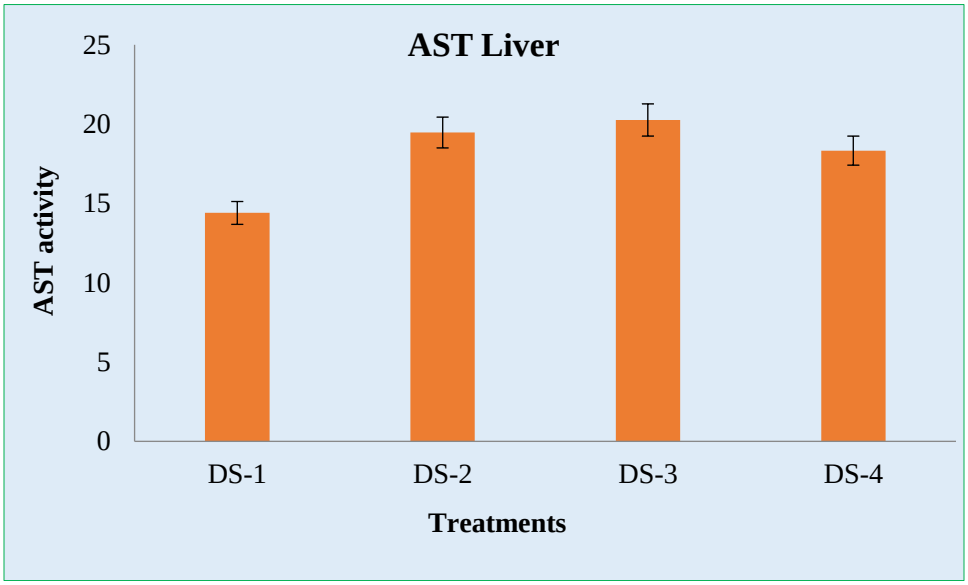

Figure 5. Aspartate amino transaminase (AST) activity of liver in the different treatment groups.

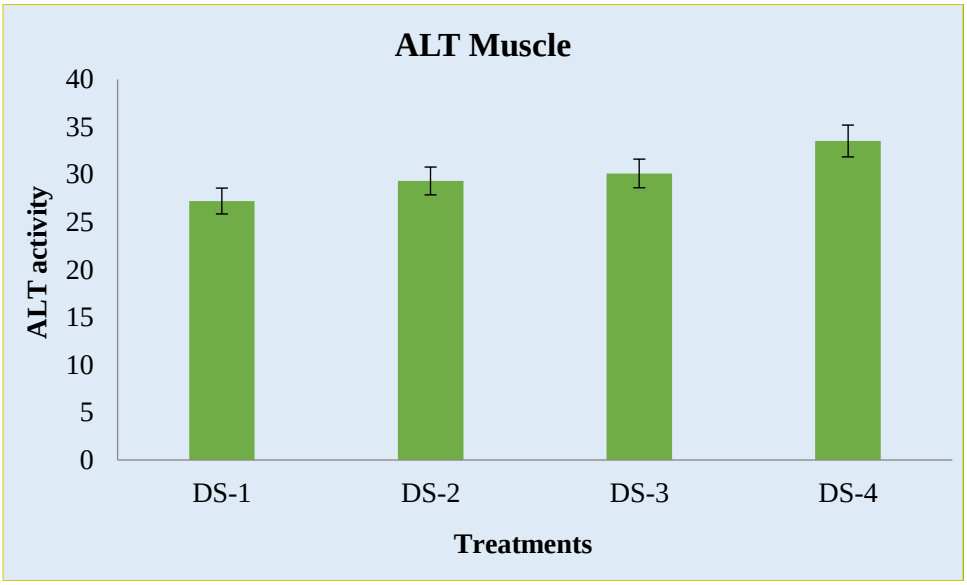

Figure 6. Alanine amino transaminase activity (ALT) of muscle in the different treatment groups.

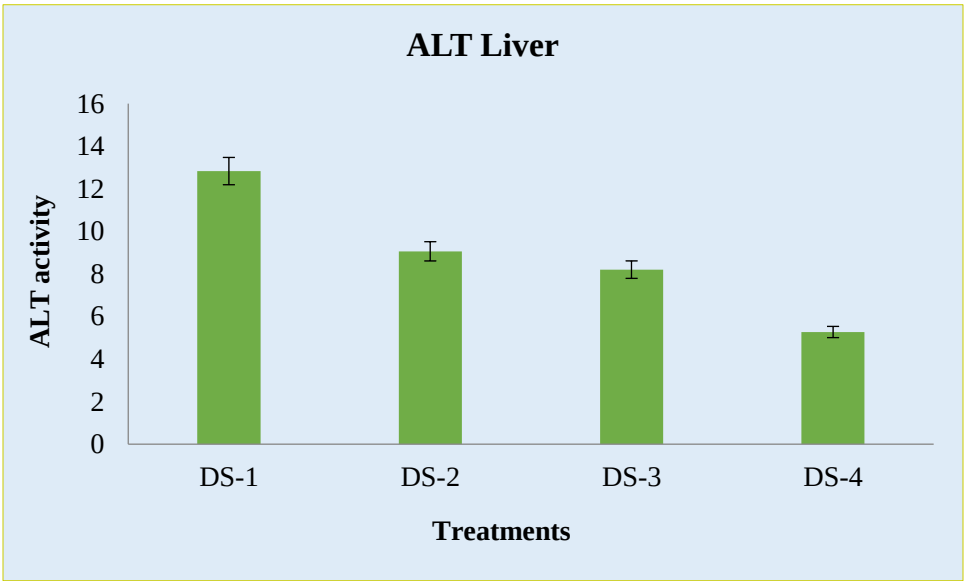

Figure 7. Alanine amino transaminase activity (ALT) of liver in the different treatment groups.

Conflict Of Interest: *The authors declare no conflict of interest.*

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