EFFECTS OF ENZYME SUPPLEMENTATION ON GROWTH AND NUTRITIONAL QUALITY OF THAI PANGAS, *PANGASIUS HYPOPHTHALMUS* (SAUVAGE, 1878)

A Thesis Submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science in Fisheries

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Dedicated to my beloved

Parents

CERTIFICATE

This is to certify that the thesis entitled"**Effects of Enzyme Supplementation on Growth and Nutritional Quality of Thai Pangas, *Pangasius hypophthalmus* (Sauvage, 1878)"** submitted by Palash Bala, has been carried out under my supervision. This is further to certify that it is an original work and suitable in partial fulfillment for the degree of MS in Fisheries, University of Dhaka.

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ABSTRACT

The present study was carried out to evaluate the effects of enzyme supplementation on the growth performance and nutritional quality Thai Pangas, *Pangasius hypophthalmus* (Sauvage, 1878) for 60 days period from May 2015 to July 2015.

The fingerlings were cultured in captive environment in eight flow-through plastic tanks (750L each) dividing them into 4 different treatment group. Aerator was used to ensure continuous aeration. Each tank was stocked with 15 fingerlings of *Pangasius hypophthalmus*. Four different types of enzymatically treated feed were used to conduct the experiment for each treatment groups. 3% enzyme mixed feed for Treatment-1, 4% enzyme mixed for Treatment-2, 5% enzyme mixed feed for Treatment-3 and feed without enzyme was used for Treatment-4 (control group).

The level of enzyme was used as the experimental variable. Effects of enzyme supplementation on growth performances such as condition factor (k), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), viscerosomatic index (VSI), hepatosomatic index (HSI), flesh yield (FY) were studied with nutritional quality (moisture, ash, protein, and lipid)assessment.

Result of the current study showed that, enzyme supplementation at a rate of 3% had significant positive effects on condition factor, SGR, FCR, VSI, FYand protein content while no significant differences were observed in ADG, HSI and ash content. However enzyme reduced moisture and lipid content at 3% rate but then increased with a dose dependent manner.Although from the study, it is found that enzyme supplementation has a very little impact on ADG, HSI, ash and lipid content, it can help in better feed production for commerciallyprofitable aquaculture.

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CHAPTER 1: INTRODUCTION

1.1 Importance and Status of Fisheries in Bangladesh

Bangladesh is land of river and it has a complex network of 230 rivers and 57 cross-boundary rivers.The rivers are not only the resource of water for fish in Bangladesh. There are so many tributaries, estuaries, long coast line, ponds, oxbow lakes and floodplain present in Bangladesh.The Bay of Bengal, the world largest bay is situated at the south of Bangladesh. As a result, the climate of Bangladesh is favorable for agriculture and fisheries resource management.

This fisheries resource is playing a very crucial role in national economy, foreign currency earning, poverty alleviation and animal protein supply. In Bangladesh, about 60% animal protein of our meal comes from fisheries resources which are composed of 260 freshwater native species, 12 species of exotic fishes, 24 species of freshwater prawn, 475 marine fish species and 36 species of marine shrimp (DoF, 2014).

Bangladesh is blessed with fisheries resources due to favorable climatic condition and geographical location compare to the other many countries. According to Department of Fisheries (DoF, 2014) the total inland aquatic resources of Bangladesh covers an area of 4,699,387 hectares which includes inland capture area or free water area (39,16,828 hectares) and inland culture area or closed water area (782,559 hectares). Inland capture area consists of river (853,863 hectares), Sundarbans (177,700 hectares), beels (114,161 hectares), Kaptai lake (68,800 hectares) and flood plains (2,702,304 hectares) where inland culture area consist of ponds and ditches (371,309 hectares), seasonal cultivation areas (130,488 hectares), baors (5,488 hectares) and shrimp farm (275,274 hectares). Besides that, Bangladesh has a vast area (166,000 sq. km) of sea in the Bay of Bengal which provides 17.27% of total fish production of Bangladesh where inland water provides the rest (82.73%). In the fiscal year of 2013-14, Bangladesh produced a total of 3,401,254 MT,contributing 4.37% in national GDP and 23.37% in agricultural GDP. The annual growth rate of the fish production in the last 5 years was 5.88%, which is likely to increase due to the growing demand for fish and fish products.

Bangladesh is one of the world's most densely populated countries with about 160 million people with high level of poverty and malnutrition (World Bank, 2010) alongside with a growth rate of 1.2%, a high rate instead. Among them 17.1 million people (about 11%) are directly or indirectly depended on fisheries sector for their livelihood. As the people of Bangladesh prefer to consume fish as a major protein source, the production is not so enough for this country because of this high population. Despite of the enormous production of fish in Bangladesh, peoples are not consuming their appropriate amount of fish. Presently the per capita annual fish consumption is 19.30kg whereas the requirement is 21.90kg (DoF, 2014).

To increase the export earning, employment possibilities, per capitaconsumption and other this necessary improvement, some tremendous goal has been set by the Department of Fisheries and other public and private institutions. Boosting the shrimp production, releasing fry in open water, beel nursery establishment, community participation, infrastructure development, enforcement of rules and regulations, stock enhancement, sanctuary establishment, breeding zone improvement and technology application are some of the points (DoF, 2014).

1.2 Potential and Status of Culture Fisheries in Bangladesh

The history of fish culture in Bangladesh in not so old. People started to use fish as a culture organisms using agricultural techniques at the last century however. Before that, the fish were harvested from the open water using various age-old techniques. But they were not available over the year to consume. So the people started to culture them in the closed area to ensure the continuous supply of fish throughout the year for their own consumption.However the small scale culture was not enough for the rapid population growth rate. To fill the demand of these vast population, aquaculture emerged as a most needed option for increasing fish production in Bangladesh. In the first time, pond culture was started for fresh water fish culture but with the increasing of demand of fish culture was begun in every water body like lakes, river, beels, haor, baor, ditch etc.Now a days, fish culture in the marine environment has become a very popular option. But in the past, tremendous effort had been given to the development of appropriate culture and seed production of different species.

Successful breeding techniques, proper management, adoption of new and appropriate technologies, public awareness, economical improvement has increased the fish production from 0.019 million MT. in 1991-92 (DoF, 2005) to 3.410 million MT in 2013-14 (DoF, 2014). Contribution of culture fisheries to the total country's yield has increased gradually from 30% in 1995-96 (DoF, 2002) to 54.54% (DoF, 2014). The massive culture process requires high supply of fry/fingerlings production which is now covered by 936 hatcheries producing 487,489 kg of fry/fingerlings per year (DoF, 2014). Bangladesh has 100 fish processing plants, where 75 of them are approved by European Union (EU) (DoF, 2014).

However the fish experts believe that the current fish production can be increased by altering the traditional extensive and semi-intensive culture to intensive culture. Moreover the water bodies of Bangladesh is not properly utilized for scientific fish culture which results in low level or per acre production than the neighboring countries. The experts also believe that, the introduction of new culture species under aquaculture practices can diversified the production in a higher rate.

Now a days, the traditional fish culture in Bangladesh is dominated by small-scale low-intensity carp production. Therefore, sometimes the culture of other large fish remains beyond the reach of the farmer of Bangladesh. But the culture of other large-scale fish can increase the fish production through which the common rural people can be benefited. Fish culture using supplementary feed is not very popular in Bangladesh. However most of the farmers use the commonly found commercial feed whereas some farmer only depends on the natural food production. Therefore the commonly available commercial feed does not contain all the ingredients that might be needed for the rapid production of the culture. The production can be increased by adding some extra ingredients to the supplementary feed.

1.3 Thai Pangas (*Pangasius hypophthalmus*) as an important fish for aquaculture in Bangladesh

Thai Pangas (*Pangasius hypophthalmus*), which is commonly known as stripped catfish, is one of the most important inland fish all over the world. In Bangladesh, 60% of animal protein comes from the fish species (DoF, 2014) where Thai Pangas is very popular in

food menu among fish, as a major source of animal protein because of its relatively low cost. To maintain the huge supply chain, it is being cultured in Bangladesh from the last three decades. Sarker (2000) reported that amongst exotic fish species Thai Pangas (*Pangasius hypophthalmus*) is the best due to its easy culture system, favorable weather condition for culture and high market demand.In recent years, Thai Pangas has become one of the most popular commercial culture-able species due to its high yield and low production cost, and many hatcheries all over the country are now producing Thai Pangas fry to meet up the farmers' demand. But still the supply is not enough for this increasing population of Bangladesh.

The last decade has seen dramatic growth in Thai Pangas production throughout much of Asia.This has been most notable in Vietnam, which produces more than 1 million tons per annumfor exporting purposes (Belton*et al.*, 2011a), but India, Myanmar, Indonesia and Bangladesh have also seen rapid expansion. Thai Pangasin Bangladesh is now by far the most important intensively cultured species in Bangladesh in volume terms (Belton *et al.*, 2011b).

This rapid growth has occurred in part because Pangasius is popular among fish farmers due to the ease with which it can be cultured; possessing hardy characteristics, good survival rates, fast growth and ability to survive at high stocking densities (Sarker, 2000).The fish has also proven popular among consumers due to its low market value, making it one of the most important cultured species, particularly among the poor in urban areas (Belton *et al.*, 2011b).

As most of the people of Bangladesh live below the poverty line, they prefer to buy a fish of low price. So the Thai Pangas has become an important fish for national food security in Bangladesh due to both the volumes produced and to its accessibility to lower income bracket consumers.Market price is low compared with that of the Indian major carp which still account for the majority of aquaculture output in Bangladesh, and which retail for approximately twice as much (Ahmed*et al.*, 2010; Belton *et al.*, 2011b; Belton*et al.*, 2012).

1.4 Effects of enzyme supplementation on the growth and nutritional quality

Enzymes are biological molecules (typically proteins) that significantly speed up the rate of the chemical reactions that take place within cells. Almost all metabolic processes in the cell need enzymes in order to occur at rates fast enough to sustain life. Like all catalysts, enzymes increase the rate of a reaction by lowering its activation energy. Thus enzyme can accelerate the growth rate and improve nutritional quality of an organism.

For the improvement of fisheries and to achieve maximum yield form resources of fresh water, it is necessary to provide artificial feed, by which fish grows rapidly and attains maximum weight in shortest possible time. Commercial fish feeds contain fish meal as the major protein source, ranging from 30-50% (Hardy, 1995). But now-a-days, fish meal is generally avoided in the feed due to its scarcity and high cost. This is of particular concern to tropical countries like Africa and Southeast Asia. Hence aquaculture nutrition has been trying to improve the nutritional value of fish feed by enzyme supplementation. Inclusion of foodstuff with relatively high levels of carbohydrate in formulated fish feed is preferred in view of its protein–sparing action that may make the diet more cost effective (Hidalgo *et al.,* 1993). According to Rumsey (1993), increased use of plant protein supplements in fish feed can reduce the cost of fish meal.

For commercial culture of fish, the formulation of low-cost balanced diet using locally available minerals, protein, and carbohydrate may need. The digestibility of all nutrients, however, including protein, carbohydrates and minerals seems to be affected (Felix and Salvaraj, 2004). Also, supplementation with enzyme can help to eliminate the effects of anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved performance of fish (Farhangi and Carter, 2007; Lin and Tan, 2007; Soltan, 2009).

The effects of exogenous and digestive enzyme supplementation in diets on growth and feed utilization of several cultured fish have been demonstrated by several researchers.

Buchanana *et al.* (1997) investigated the effects of additional enzyme with canola meal on prawn (*Penaeus monodon*). The results showed that the enzyme mixed canola based diet resulted a significantly higher weight gain but no change in FCR.

Devnath *et al.* (2005) experimented with 7 isocaloric (3870 kCalKg^{-1}) and isoprotein (35.67% CP) experimental diets which were prepared with graded levels of phytase (0, 150, 250, 350, 500, 1000 and 2000 FTU [phytase units]Kg^{-1}) to estimate the effect on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Maximum weight gain (350.72%), specific growth rate (2.51%), protein efficiency ratio (2.1), apparent net protein utilization (27.85%), energy retention value (88.47%) and feed conversion efficiency were observed in the group supplemented with 500 FTU phytaseKg^{-1}) diet. Apparent dry matter and protein digestibility in phytase-supplemented groups were significantly (P<0.01) higher at a minimum supplement of 500 FTU Kg^{-1} or higher.

Farhangi and Carter (2007) found a negative relation in their experiment on rainbow trout, *Oncorhynchus mykiss*. High inclusion levels of de-hulled lupin in salmonid diets significantly decrease growth rates. The supplemented enzymes did not affected digestive tract indices or carcass composition.

Jackson and Robinson (1996) conducted a feeding trail on channel catfish *Ictalurus punctatus*, fed with five different units of microbial phytase/kg diet. Fish fed diets containing 500 or more units of microbial phytase/kg consumed more feed and gained more weight than fish fed the basal diet without supplemental phytase. Contrast analysis showed that weight gain, feed consumption, bone ash, and bone phosphorus were higher and feed conversion ratio was lower for fish fed diets supplemented with phytase as compared to fish fed no supplemental phytase. The concentration of fecal phosphorus decreased linearly as phytase supplementation increased. Results from this study demonstrate that microbial phytase is effective in improving bioavailability of phytate phosphorus to channel catfish, which may eventually lead to a reduction in the amount of supplemental phosphorus added to commercial channel catfish feeds.

Kamruddin *et al.* (2011) found that, the*Mystus nemurus* larvae which fed on Artemia nauplii gave the highest survival rate (83.7%) and growth rate followed by those fed on a

combination diet (56.0%) and a microbound diet (26.5%). This suggested the important role of exogenous enzymes from live food in the larval digestion particularly at the early feeding stages.

Kolkovski *et al.* (1993) found that, the exogenous enzyme supplementation improved the nutritional value of feed which had a positive impact on the growth rate of gilthead seabream (*Sparus aurata*).

Siti-Norita *et* al. (2015) conducted a study to evaluate the influence of β-mannanase supplementation on the growth performance, apparent nutrient digestibility (ADCs), meat and carcass content in tilapia fed palm kernel cake (PKC). β-Mannanase supplementation led to an increase ($P<0.05$) in the ADCs of crude protein, ash and fiber compared with the control. The results suggested that the supplementation of diet containing PKC with β-mannanase could improve the growth performance, energy, and nutrient digestibility of tilapia, thus increasing the nutritional value of PKC as potential feed ingredients.

Sayeed *et al.* (2008) undertook a research to assess the effect of three types of feed with different protein level on growth of Thai pangus (*Pangasius hypophthalmus*) and rohu (*Labeo rohita*) in polyculture system. The result showed a typical increasing trend of final weight and specific growth rate of Thai Pangas along with the increasing of feed protein level.

Tahoun *et al.* (2011) conducted a study to investigate the effects of a commercially exogenous enzyme (AmecoZyme 2X[®]) supplementation at a rate of 0.5g/kg and/or inclusion of 5% fishmeal on reproductive performance of Nile tilapia (*Oreochromis niloticus*). They found that the supplementation of AmecoZyme 2X® at 0.05% and/or inclusion of 5% fish meal improved reproductive parameters values significantly while total seed production, absolute fecundity, relative fecundity and system productivity followed the same trend.

Yiğit *et al.* (2014) experimented the effects β-mannanase and α-galactosidase enzymes on growth performances, feed efficiency and nutrient digestibility of Rainbow trout (*Oncorhynchus mykiss*). The result showed that the β-mannanase, α-galactosidase mixed diet had no significant effects on growth, nutrient digestibility ($p>0.05$).

Yildirim and Turan (2010) studied on the effects of a multi enzyme complex containing fungal xylanase, hemicellulose, cellulose, β-amilase, β-glucanase, pentosonase, fungal β-glucanase, cellubiase and pectinase on African Catfish, *Clarias gariepinus*. Growth rate was significantly increased in fish fed with enzyme complex supplemented diets in comparison with the control groups. The best specific growth rate and highest value of protein content (21.75%) was observed at $0.75gKg^{-1}$ enzyme complex group.

Zamini *et al*. (2014) studied the effects of two supplemental exogenous enzymes (Natuzyme and Hemicell) on the growth performance in Caspian salmon (*Salmo trutta caspius*) over an 8-week feeding trial. Significantly higher growth rate, best feed conversion ratio (0.64±0.01), higher White Blood Cell count (7,716.67 ± 348.80 N/mm3) was observed in the group receiving NH (0.5 g Natuzyme$^{®}$Kg^{-1} + 0. 5 g Hemicell$^{®}$Kg^{-1}) feed than the control group. The results suggested that enzyme supplementation caused significant improvement on growth performance and feed utilization in Caspian salmon.

1.5 Rationale

Feed is the single most important item for the viability and success of aquaculture particularly in terms of feed cost. Poor fish feed quality and nutrition are the two major impediments in the fields of fish culture around the world including Bangladesh. The poor quality feed decreasing the production in the country like Bangladesh. So it is much needed to introduce quality feed in the field of fish culture to utilize the resources properly in Bangladesh. Moreoverfeed should be used optimally to prevent the input of more nutrients than necessary. To avoid the waste of feed ingredients should increase the efficiency of food use and to reduce production costs. Enzyme can be used to improve the feeding efficiency. Feed cost is one of the largest operational costs in aquaculture. Therefore, enzyme supplementation can be pointed as one of the most important element in the culture practice. So, it is assumed that this supplementation will increase growth performance and nutritional quality of Thai Pangas.

1.6 Research Need

Some literatures are available on the supplementation of different exogenous enzyme on different fish; *Mystus nemurus* (Kamruddin *et al.,* 2011);seabream, *Sparus aurata* (Kolkovski *et al.,*1993); phytase on *Pangasius pagasius* (Devnath *et al.*, 2005) and*Ictalurus punctatus*(Jackson and Robinson (1996); AmecoZyme 2X®on Nile tilapia, *Oreochromis niloticus* (Tahoun *et al.,* 2011);β-mannanase and α-galactosidase on Rainbow trout,*Oncorhynchus mykiss* (Yiğit *et al.,*2014);multi enzyme complex(xylanase, hemicellulose, cellulose, β-amilase, β-glucanase, pentosonase, fungal β-glucanase, cellubiase and pectinase) in African Catfish, *Clarias gariepinus*(Yildirim and Turan 2010)*;* Natuzyme and Hemicell on *Salmo trutta caspius* (Zamini *et al*., 2014).

While Sayeed *et al.* (2008) undertook a research to assess the effect of three types of feed with different protein level on growth of Thai Pangas (*Pangasius hypophthalmus*) and rohu (*Labeo rohita*) in polyculture system. However, effects of enzymes on the growth performance such as average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), flesh yield (FY), condition factor (k), hepatosomatic index (HSI), viscerosomatic index (VSI), protein, moisture, lipid and ash content of Thai Pangas (*Pangasius hypophthalmus*) has not been reported.Therefore, this study evaluated the effects of exogenous enzyme on the growth performance such as average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), flesh yield (FY), condition factor (k), hepatosomatic index (HSI), viscerosomatic index (VSI), protein, moisture, lipid and ash content of Thai Pangas (*Pangasius hypophthalmus*).

1.7Objectives

The objective of this research is to determine the effects of enzyme supplementations on growth and nutritional quality of Pangas (*Pangasius hypophthalmus*) fingerlings.

The specific objectives are:
1. Determination of growth performance such as Condition factor, average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR),Viscerosomatic Index (VSI), Hepatosomatic Index (HSI) and Fillet Yield (FY) of Pangas (*Pangasius hypophthalmus*) of different treatments;

2. Determination of Moisture, Ash, Crude Protein and Crude Lipid of fish flesh and carcass composition of Pangas (*Pangasius hypophthalmus*)of different treatments.

CHAPTER 2: MATERIALS AND METHODS

2.1 Description of the experimental site

The experiment was carried out in the Aquatic Laboratory of Department of Fisheries, University of Dhaka. The experiment consisted of various steps in an organized sequence. Feeding trial of the experimental fish with prepared feed in eight tanks was done for 60 days in the laboratory.

2.2 Experimental fish and its taxonomic position

The striped catfish, *Pangasius hypophthalmus* (Sauvage, 1878), also known asriver catfish or sutchi catfish (Paripatananont, 2002; Rahman *et al*., 2006) and Thai Pangas locally, is a fast growing omnivorous fish and tolerant to high stocking densities was used as the experimental species for this study.

Kingdom: Animalia

Phylum: Chordata

Class: Actinopterygii

Order: Siluriformes

Family: Pangasiidae

      Genus: *Pangasius*

Species: *P. hypophthalmus*

2.3 Collection of the experimental fish and acclimationin the laboratory

The fingerlings (Avg. length 9.20cm, Avg. weight5.96 g) of *Pangasius hypophthalmus*wereobtained from ahatchery of Mymensingh, Bangladesh. Conditioning of these fish was done for three days in the laboratory condition. No feed was given in the conditioning period to evacuate their previous gut content fully. After conditioning, dead ones were culled from the conditioning tank while fresh looking healthy fish with similar size and weight was selected for the experiment. Length (cm) and weight (g) of 120 fish was measured and randomly grouped into eight groups to stock in eight tanks.

2.4 Experimental enzyme and replication

Zymex, a commercially available multi-enzyme complex (Amylase, Xylase, Protease, B. Glucanase, Phytase and Pectinase) was used for this experiment to estimate the effect on the experimental fish. Three different concentrationof enzyme complex was used in the feed for three different groups of fish where they were called Treatment-1, Treatment-2, Treatment-3 and a group was treated with enzymatically untreated feed which was called as Control group. Duplication of these four treatment group was established in the laboratory to minimize the statistical error.

2.5Experimental design

The fingerlings were cultured in captive environment in eight flow-through plastic tanks (750L each). There was three experimental tanks for three different enzymatically treated feed and a control tank for enzymatically untreated feed where the left four tanks was the replica of the total experiment. Aerator was used for the continuous aeration. Eight plastic tanks were filled with tape water and placed inside the Aquatic Laboratory of Department of Fisheries, University of Dhaka. Water exchange was done in every morning during the whole experiment period to avoid the decomposition of fish excreta.Previously recorded random group of*Pangasius hypophthalmus* fish was released in those eight tanks.They were given theexperimental feed twice a day at 5% body weight ratio. Feeding schedule was9am at morning and 5pm at evening.Weight of the given feed in every tank was recorded in each time to determine the weight gain ratio. Abiotic parameters of the tanks such as pH, DO, temperature was monitored and maintained during the experiment. Individual weight of every fish was measured and recorded at the end of the month. The collection and analysis of these information gathered using this experimental design mentioned above helped the evaluation of growth performance, ADG, FCR, SGRof the specimen.

Sample fish were taken and eviscerated at the first step of the experiment to evaluate the viscerosomatic index (VSI), hepatosomatic index (HSI), fillet yield(FY), condition factor (k), length-weight relationship. Same was done at the end of the 60 days trial period to compare with the initial one.

2.6 Collection of feed

A locally available commercial feed manufactured by BRAC enterprises was used for this experiment. Grower feed from BRAC enterprises feed mill was bought to the Aquatic Laboratory of Department of Fisheries, University of Dhaka to feed the experimental specimen for 60 days.

2.7 Proximate composition of feed

Proximate composition of the collected commercial feed from BRAC was evaluated at the initial stage of the experiment. Moisture, Ash and Protein content was determined by the A.O.A.C method (1990) and Lipid content was determined with the automatic lipid extractor machine. Table 2 shows the proximate composition of the feed.

2.8 Preparation of experimental feed

Four types of experimental feed was prepared with different enzyme content for four treatment group.Enzyme complex was mixed with the feed at a different concentration. 3%, 4% and 5% where enzyme mixed feed was called Treatment-1, Treatment-2 and Treatment-3 respectively while a Control group was also established to evaluate the normal growth behavior where no enzyme was added with the feed. Addition of the enzyme was done with micro pipette (Plate 1).After the well mixing of enzyme with a stick (Plate 2), the feed was dried in normal temperature to let it combined with the feed properly. Table 1 shows the enzyme percentage added in the feed of different treatment group.

Table 1: Amount of enzyme mixture for different types of feed.

Treatment Group	Percentage (%) of enzyme added to the feed
Control Group	0
Treatment-1	3
Treatment-2	4
Treatment-3	5

2.9 Recording of initial body indices of fish

Five sample fish were taken from the collected *Pangasius hypophthalmus* fish were randomly taken to evaluate the initial viscerosomatic index (VSI), hepatosomatic index (HSI) and fillet yield (FY).

2.10 Recording of initial condition factor

120 fish randomly taken from the collected batch. Then they were grouped into eight group.The length (Plate 8) and weight (Plate 9) of each fish of each group were taken and recorded. Average length, weight were taken and condition factor were calculatedfor each group. These eight group were used as the initial population of eight different tanks.

2.11Proximate composition of the experimental fish

After the calculation of body composition and length-weight relationship, the proximate composition of the *Pangasius hypophthalmus* fish were determined at the initial stage of the experiment. Moisture, Ash and Protein content was determined by the A.O.A.C method (1990) and Lipid content was determined with the automatic lipid extractor machine.

2.12 Feeding trial of fish

2.12.1 Establishment and arrangement of experimental tanks and equipment

Eight flow-through cleaned plastic tanks (750L each) were established in the Aquatic Laboratory of Department of Fisheries, University of Dhaka. Then they were filled with tape water up to 500L mark.Aerator was placed for the continuous aeration. They were randomly divided into four groups. Tanks of each group were sealed with paper and tape denoting the number treatment group; such as Treatment-1, Treatment-2, Treatment-3 and Control group. A duplicate treatment was carried out for each of these treatment. Water exchanging option was also ready for each tank by aerator. Plate 3 shows the arrangement of the tank inside the laboratory.

2.12.2 Fish stocking in tank

To each of the experimental tanks,one previously grouped fish of Pangas fingerlings were released at the first day of this experiment.The releasing of the fingerlings in the tank was done so gently to avoid any kind of injury. Plate 4 shows the releasing of fish in the tank with great handling care.

2.12.3 Feeding schedule and frequency

After the collection of experimental *Pangasius hypophthalmus* fingerlings, preparation of feed, setup of experimental tanks, stocking them in those tanks, the feeding trail was initiated. They were given enzymatically treated feed at 5% body weight ratio. The weight was taken with an electronic balance (Plate 5). As theweight of each fish group was measured at the initial step of this experiment, the amount of feed was calculated from those data. Feed were given directly to the tank (Plate 6).Feeding schedule was twice a day; 9am at morning and 5pm at evening. Weight of the given feed in every tank was recorded in each time to determine the weight gain ratio. Fish weight and length were recorded to find out the biological parameters such as FCR, ADG, SGR, condition factor at the end of the 30 day and 60 day rearing period.

2.12.4 Water exchange and aeration

Tanks are supplied with fresh water from overhead tank through steel pipe. For proper aeration of the water, aerators were fitted in each tank. To ensure water quality and safety of fish, the water of the tanks were changed for each other day during the experimental time.

2.12.5 Cleaning of tanks

Every tank was cleaned once in a week to completely remove the excreta, uneaten feed and the other materials from the bottom. A brush was used to brush the wall of the tank and running water from a plastic tube was forcedly introduced to the tank to wash them properly. Manual movement of those tank was done during the washing. Plate 7 shows the cleaning procedure at a glance.

2.13 Study of the fish growth

2.13.1 Fish sampling procedure

Sampling was accomplished at 30 and 60 days of the experiment period. Prior to the experiment, the fishes were captured with a fine mesh scoop net. Then their individual length and weight were measured to the closest centimeter and closest gram respectively.

After 60 days of rearing period or at the termination of the experiment, the final length (cm)and weight (g)of the individual fishes were carefully recorded. A wooden measuring scale was employed for measuring the lengths (Plate 8)while an electric balance (Plate 9) was used to determine the individual fish body weight.

2.13.2 Analysis of experimental data

The experimental data which were collected before, during or after the experiment period were used to determine the following growth parameters and nutritional quality.

2.13.2.1 Condition factor (k)

The condition factor is an expression of the condition of the fish in numerical terms i.e. degree of plumpness or fatness is usually estimated as the condition factor. It was calculated by the following formula-

$$K = \frac{W}{L^3} \times 100$$

Where,

K = Condition Factor,

W = Body weight it grams,

L = Body length in centimeters.

2.13.2.2 Average daily gain (ADG)

Average daily gain (ADG) is simply the rate of weight gain per day over a specified period of time. It was determined by the following formula-

$$ADG = \frac{\text{Meanfinalfishbodyweight-Meaninitialfishbodyweight}}{\text{Time } (T_2\text{-}T_1)}$$

Where,

T_1 = Initial time,

T_2 = Final time.

2.13.2.3 Specific growth rate (SGR %)

The specific growth rate is dependent on the number of factors including species, age, genetic potential, water temperature, health, quantity and quality of food. The simplest modes for fish growth can be obtained by saying that all newly laid-down tissue is itself capable of equal growth thereby producing an exponential growth curve.

However this only holds true if the percentage of body weight gained per unit times remains constant throughout the life of the fish. This is not the case young fish are capable of doubling their weight in a much shorter time than when they are older due to a decrease in potential growth rates. It is therefore useful to be able to ascertain the rate at which fish are growing. The best method of doing this is to calculate the specific growth rate (SGR), which is a measure of the percentage of body weight increase per day.

Specific growth rate (SGR) was calculated as the percentage increase in weight per animal per by the following formula-

$$SGR\ \% = \left\{ \frac{\ln W_T\text{-} \ln W_t}{(T\text{ - }t)} \right\} \times 100$$

Where,

SGR % = Percentage increase in body weigh per fish per day,

$\ln W_T$ = Natural log of weight at time T,

$\ln W_t$ = Natural log of initial weight,

T = Time T,

t = initial time.

At first feeding, SGR % can be greater than 3 while fish over 1.0 kg have average values of 1. This is because smaller fish are capable of eating a much greater percentage of their body weight per day. Temperature also has an effect on the SGR- the higher the temperature, the higher the growth rate. According to the fifth law of Medawar (1945), the specific growth declines more and more slowly as the organism increases in age. Minot (1908) was the first person to recognize that for most animals the specific growth rate is highest early in life and that is typically decreases with increasing age, becoming zero in some animals and his epigram. "Organism age fastest, when try are young is repressed Medawar's fifth law.

2.13.2.4Feed conversion ratio (FCR)

Feed conversion ratio (FCR) was calculated from the number of Kg's of feed that are used to produce one Kg of whole fish. Food conversion ratio (FCR) was determined by the following formula:

$$FCR = \frac{\text{Feed consumed by the fish (g)(dry weight basis)}}{\text{Live weight of the fish (g)}}$$

Commercially available pelleted diets usually give FCR between 1 to 3 depending on the fish size, temperature, feeding rate and feed composition. FCR of 1 means that every Kg of feed fed is converted to 1 Kg of fish flesh.

Farms reporting a low FCR generally have good management practice in place, with no overfeeding or underfeeding and very low, if any, mortalities. Over feeding or under feeding will increase the FCR. In order to calculate FCR, several things need to be measured, calculated and understood. It also requires the maintenance of good, accurate records whether they be paper or computer generated. Without these, the calculations are impossible. The FCR can range from 1.5 to 2.0. The lower the FCR the better the feed conversion to fish flesh.

2.13.2.5 Viscerosomatic Index (VSI %)

Viscerosomatic index is a numerical presentation of the viscera weight in proportion with the body weight. It actually defines the percentage of body weight equal to the viscera. Three sample fish from each treatment tanks was taken randomly to determine the viscerosomatic index. The body weight was taken first and then the fish were eviscerated to extract the viscera from them. Then viscerosomatic index was calculated with the following formula:

$$\text{Viscerosomatic Index (VSI \%)} = \frac{\text{Viscera weight}}{\text{Body Weight}} \times 100$$

2.13.2.6 Hepatosomatic Index (HSI %)

Hepatosomatic index is a numerical presentation of the liver weight in proportion with the body weight. It actually defines the percentage of body weight equal to the liver. Three sample fish from each treatment tanks was taken randomly to determine the viscerosomatic index. The body weight was taken first and then the fish were eviscerated to extract the viscera from them. Then viscerosomatic index was calculated with the following formula:

$$\text{Hepatosomatic Index (HSI \%)} = \frac{\text{Liver weight}}{\text{Body Weight}} \times 100$$

2.13.2.7 Flesh Yield (FY %)

Flesh yield is a numeric expression of the total flesh weight in proportion with the total body weight. Three sample fish were taken randomly from each of the treatment tanks to determine the flesh yield.The body weight was taken first and the fish were eviscerated to collect all the flesh from the body without the guts and viscera. Then flesh yield was calculated with the following formula:

$$\text{Flesh Yield (FY \%)} = \frac{\text{Flesh weight}}{\text{Body weight}} \times 100$$

2.13.2.8 Moisture (%)

Moisture content is expressed as the amount of water as a percentage. It was determined by oven drying method (A.O.A.C., 1990) (Plate 10). Pre weighted samples were oven dried using pre weight porcelain cups. Moisture content determined as the loss of weight.

$$\text{Moisture}(\%) = \frac{(W_1 - W_0) - (W_2 - W_0)}{(W_1 - W_0)} \times 100$$

Where,

W_0 = Weight of clean, dry crucible,

W_1 = Weight of clean, dry crucible + wet sample,

W_2 = Weight of clean, dry crucible + dry sample.

2.13.2.9 Ash (%)

Ash content was determined by ignition of samples in a muffle furnace at 550°C for 16 hours (Plate 11). Then the following formula was used to determine the value.

$$\text{Ash } (\%) = \frac{W_2 - W_0}{W_1 - W_0} \times 100$$

Where,

W_0 = Weight of clean, dry crucible,

W_1 = Weight of clean, dry crucible + dry sample,

W_2 = Weight of clean, dry crucible + dry sample + ash.

2.13.2.10 Crude protein (%)

Protein is the most important constituent from the nutritional point. Proteins are highly complex nitrogenous organic substances of very high molecular weight. Proteins are the polymers of amino acid that contain carbon, hydrogen, oxygen, nitrogen and sulphur. Protein content in most fish average 18-20%. Protein content may vary considering age, fat content, spawning and starvation etc.

Crude protein content was determined by Kjeltec machine (Model Tecator Kjeltec System) (Plate 12). The principle of this method for the determination of protein is based on the conversion of nitrogen of protein into $(NH_4)_2SO_4$ and amines when digested with H_2SO_4 which on distillation with excess of sodium hydroxide liberates ammonia which is then absorber in boric acid solution with indicator. By titration with N/100 Hydrochloric acid (HCL) solution, the amount of nitrogen absorbed in boric acid is determined directly. The nitrogen value is then multiplied by protein conversion factor.Protein conversion factor (6.25) was used in converting nitrogen to crude protein. The percentage of Nitrogen in the sample was calculated by using the following formula.

$$\text{Nitrogen Content (\%)}= \frac{(S - B) \times A \times N \times C \times 100}{W \times 1000}$$

Where,

S = Burette reading,

B = Burette reading of blank sample,

A = Atomic mass of Nitrogen,

N = Strength of Acid, (N=0.01 in this experiment),

C = Dilution factor ($C= \frac{100}{5} = 20$, in this experiment),

W = Sample weight.

And the protein (%) was calculated by the following formula:

Protein (%)= N_2(%) × Protein conversion factor × Moisture factor

Where,

Protein conversion factor = 6.25,

Moisture factor = $\frac{(100 - \text{Moisture})}{100}$

2.13.2.11 Crude Lipid (%)

Crude lipid was determined by the automatic lipid extractor (Soxtec system) (Plate 13). Sample of about 3 grams were taken in anextraction cup and 40ml of n-hexane was added the vessel.Then the extraction cup was set with the magnet of thimble where the

vessel was placed under the thimble. After that, the extraction cup was emerged to the vessel into the n-hexane solvent andgiven to be boiled at 100-110°C roughly.After the specified boiling duration, the thimbles were raised out of the solvent and the rinsing operation was initiated. During rinsing, the evaporated solvent from the extraction cups condensed when contacting the condensers, which had cooling water (≈20°C) running through them. After the rinsing period over, the lipid then deposited in the vessel. The following formula was used to determine the lipid:

$$\text{Lipid (\%)}= \frac{W_2 - W_0}{W_1}$$

Where,

W_0 = Initial vessel weight,

W_1 = Sample weight,

W_2 = Final weight of vessel.

2.14 Statistical Analysis

Mean final body weight (BW), specific growth rate (SGR), feed conversion ratio (FCR), average daily gain (ADG), condition factor (k), moisture, ash, protein and lipid data were transformed into square root transformations before analysis. Difference between treatments were compared by using 1-way ANOVA with Tukey's HSD post hoc for multiple comparison. Statistical software SPSS version 20.0 was used to analyze data. Any significant difference was determined at P < 0.05 level. Normality and homogeneity of variance (Bartlett's test) was verified prior to the analysis (Sokal and Rholf, 1981). Second-order polynomial regressions was used to derive the relationships between SGR and feeding rate as well as FCR and feeding rate.

CHAPTER 3: RESULTS

This study had two aspects: growth performance determination and nutritional quality assessment of Thai Pangas (*Pangasius hypophthalmus*). The data was collected at the initial stage of the experiment, after 30 days of the experiment and at the last day of the experiment (after 60 days). Proximate composition of collected commercial feed also done. Table 2 shows the composition value of the feed. Detailed result of the study on the growth performancenutritional quality of the experimental fish in eight tanks fed on three enzymatically treated (3%, 4% and 5% enzyme mix feed) and a non-enzymatically treated (no enzyme mixed) diet for 60 days experimental period including the initial recordsare presented below where Table 3 shows the initial body indices, Table 4 shows the initial length-weight relationship and condition factor (k), Table 5 shows the body indices after 30 days, Table 6 shows the body indices after 60 days, Table 7 shows the initial proximate composition and Table 8 shows the final body composition of the experimental fish.

3.1 Growth Performances

There are several parameters which could be observed as a growth performance indicator. In this study, condition factor (k), average daily gain (ADG), specific growth rate (SGR), feed conversion ratio (FCR), viscerosomatic index (VSI), hepatosomatic index (HSI) and flesh yield (FY) was observed as growth performance indicator. Some author calls it feed utilization efficiency.For condition factor, average daily gain, specific growth rate and feed conversion ratio, the initial record was compared to the final record along with the comparison of 60 days rearing period report with 30 days rearing period. On the other hand, viscerosomatic index, hepatosomatic index and flesh yield were compared only with the final record of treatment tanks in this experiment to see the best effect of different diet feeding.

Table 2: Proximate composition of the collected feed from BRAC feed mill

Parameters	Percentage (%)
Crude Protein	33.10
Crude Lipid	6.01
Ash	16.69
Moisture	8.45

Table 3: Initial body indices of the experimental fish, *Pangasius hypophthalmus*.

Composition Parameter	Percentage (%)
Hepatosomatic Index (HSI)	10.79
Viscerosomatic Index (VSI)	2.19
Fish Yield (FY)	34.84

Table 4: Initial length-weight relationship of *Pangasius hypophthalmus*.

Fish group	Avg. Length (cm.)	Avg. Weight (g)	Condition Factor (CF)
Fish group 1	9.05	6.48	0.867
Fish group 2	9.25	6.02	0.762
Fish group 3	9.29	5.86	0.732
Fish group 4	8.85	5.08	0.733
Fish group 5	9.21	6.60	0.846
Fish group 6	9.65	6.45	0.717
Fish group 7	9.34	5.92	0.727
Fish group 8	8.95	5.28	0.735

Table 5: Body indices of fish growth after 30 days rearing period.

Treatment	Replication	K	ADG	SGR (%)	FCR
Control	1	0.733	0.149	1.625	2.771
	2	0.735	0.201	1.504	2.865
Treatment -1	1	0.876	0.172	1.720	2.220
	2	0.846	0.199	1.673	2.251
Treatment -2	1	0.762	0.193	1.499	2.873
	2	0.717	0.212	1.256	2.857
Treatment -3	1	0.732	0.197	1.288	2.985
	2	0.727	0.152	1.421	2.958

Table 6: Indices of fish growth after 60 days rearing period.

Treatment	Replication	K	ADG	SGR (%)	FCR	VSI (%)	HIS (%)	FY (%)
Control	1	0.744	0.178	1.515	2.785	8.60	2.27	47.80
	2	0.731	0.205	1.331	2.834	8.62	2.30	47.46
Treatment -1	1	0.884	0.207	1.670	2.283	8.23	2.40	48.25
	2	0.867	0.244	1.577	2.320	8.28	2.41	48.87
Treatment -2	1	0.804	0.208	1.352	2.884	8.47	2.38	47.27
	2	0.754	0.191	1.182	2.891	8.42	2.35	47.31
Treatment -3	1	0.744	0.234	1.361	2.973	8.99	2.41	46.54
	2	0.749	0.161	1.372	2.968	8.98	2.40	46.63

3.1.1 Condition Factor (k)

Condition factor (k) was measured at the first day of the rearing period (Table 4) and at the end of the 60 day rearing period (Table 6). The highest (0.876±0.009) condition factor (k) was obtained in the Treatment-1 group fed with 3% enzyme mixed feed and the lowest (0.738±0.007) was obtained in the control group fed withenzymatically untreated feed. The condition factor of Treatment-1 was significantly different than other treatment groups.On the other hand,Treatment-2 (0.778±0.025), Treatment-3 (0.747±0.003) and control group (0.738±0.007) showed a similar pattern of condition factor without any significant differences.However there was no significant differences within the group at different rearing period(Figure 1).
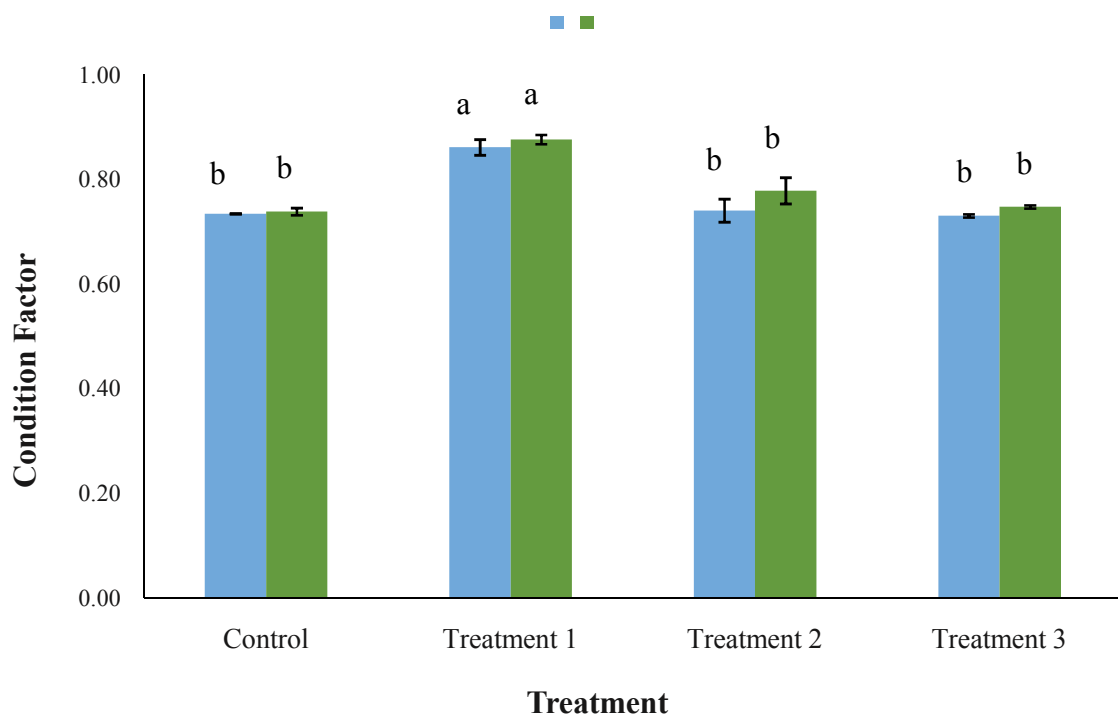
Figure 1: The condition factor (k) comparison between initial and 60 day rearing period of the *Pangasius hypophthalmus* in different treatment method. Data are represented as Mean ± SEM. Bars with same color different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.1.2 Average Daily Gain (ADG)

Average daily gain (ADG) was calculated after 30 days of rearing period (Table 5) and after 60 days of rearing period (Table 6). The highest (0.225±0.020) average daily gain (ADG) was observed in the Treatment-1 group where 3% enzyme mixed feed was supplemented while the lowest (0.195±0.040) was observed in the Treatment-3 group. However the control group showed the exact same value (0.195±0.202) of ADG as Treatment-3 but with different SEM (standard error mean). AgaintheTreatment-2 showed an average value (0.200±0.010). Somehow all the value of ADG for all these treatments were close to each other with no significant difference. Even no significant difference was found within a single treatment after 30 days and 60 days rearing period (Figure 2).
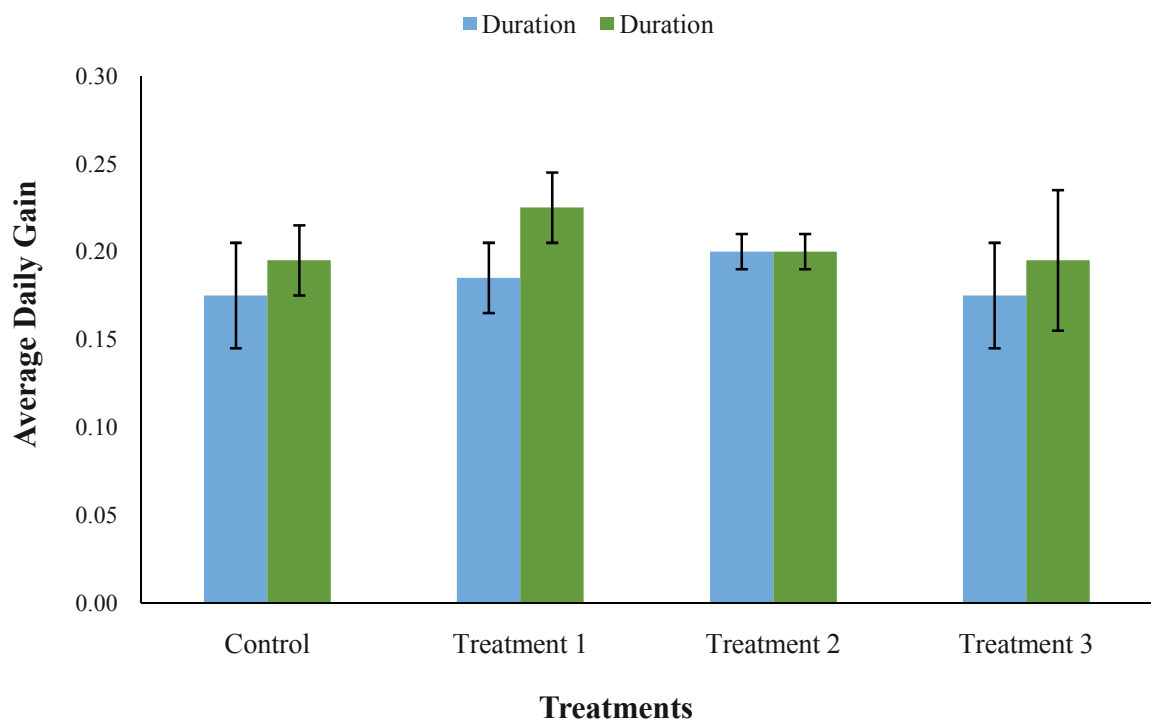
Figure 2: The average daily gain(ADG) comparison between 30 day and 60 day rearing period of the *Pangasius hypophthalmus* in different treatment method. Data are represented as Mean ± SEM.

3.1.3 Specific Growth Rate (SGR)

The specific growth rate (SGR) were calculated after 30 days of rearing period (Table 5) and after 60 days of rearing period (Table 6). The highest (1.6237± 0.05) specific growth rate (SGR) was found in Treatment-1 group where 3% enzyme mixed supplemented feed was given, where the lowest (1.2621± 0.08) specific growth rate was found in Treatment-2.The other treatments (Treatment-3 and control) showed a similar pattern in specific growth rate. However the difference among them and within rearing period was not significant. But the treatment-1 showed two significantly different SGR at 30 days and at 60 days rearing period. Again the treatment-2 group showed a similar pattern of significant result after 30 days and 60 days rearing period (Figure 3).
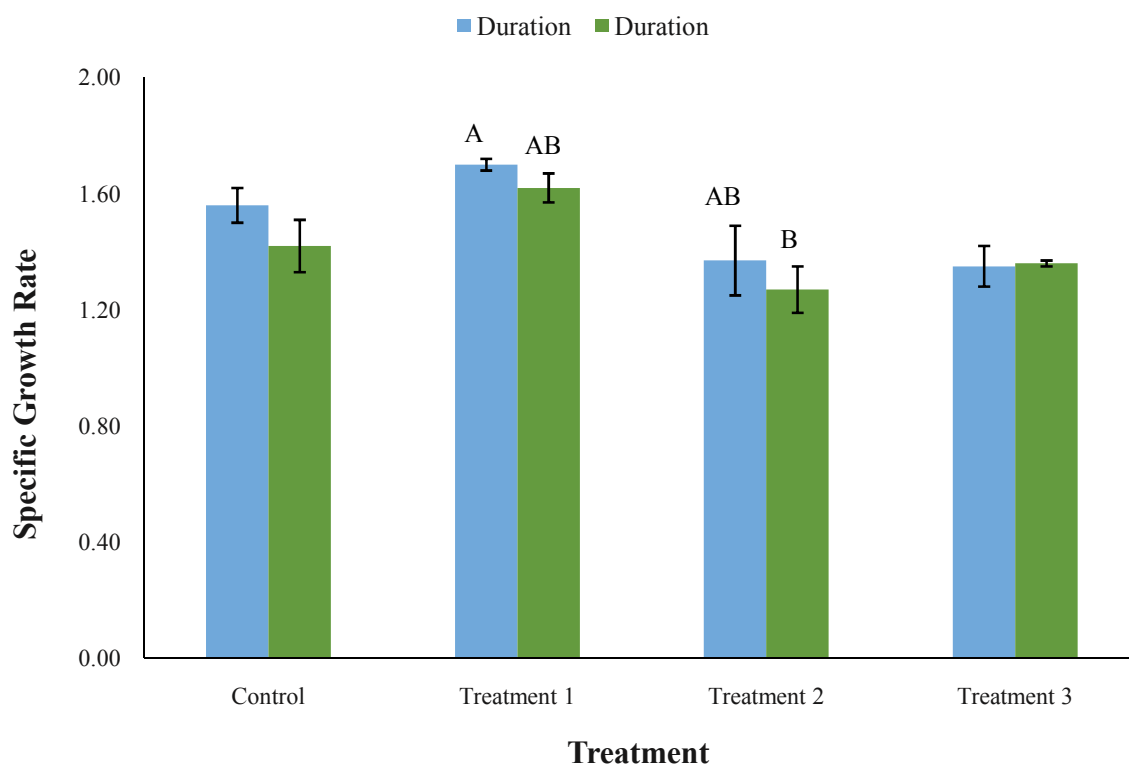
Figure 3: The specific growth rate (SGR) comparison between 30 day and 60 day rearing period of the *Pangasius hypophthalmus* in different treatment method. Data are represented as Mean ± SEM. Bars with same color different letters are significantly different (p<0.05, Tukey, LSD).

3.1.4 Feed Conversion Ratio (FCR)

The feed conversion ratio (FCR)were calculated after 30 days of rearing period (Table 5) and after 60 days of rearing period (Table 6). The lowest and best Feed Conversion Ratio (2.236$\pm$ 0.019) was found in the treatment-1 where the fish were fed with 3% enzyme mixed feed and the highest (2.971 $\pm$ 0.003) FCR was found in the Treatment-3 groups where 5% enzyme mixed feed was given, compared to the other groups after each 30 days rearing interval. The FCR observed within a single treatment was almost same after 30 days and 60 days rearing period where the difference was not significant. But different FCR was found in different treatment group at the end of each month rearing period. However significant difference in FCR was observed in Treatment-1 than the other treatments after 30 days and 60 days rearing period as well. Treatment-2 showed a similar pattern of FCR with control group where no significant difference was found in any duration. Also the Treatment-2 and Treatment-3 showed similar result with no significant difference. However the FCR of treatment-3 was higher than the control group which was significant after both 30 days and 60 days rearing period (Figure 4).
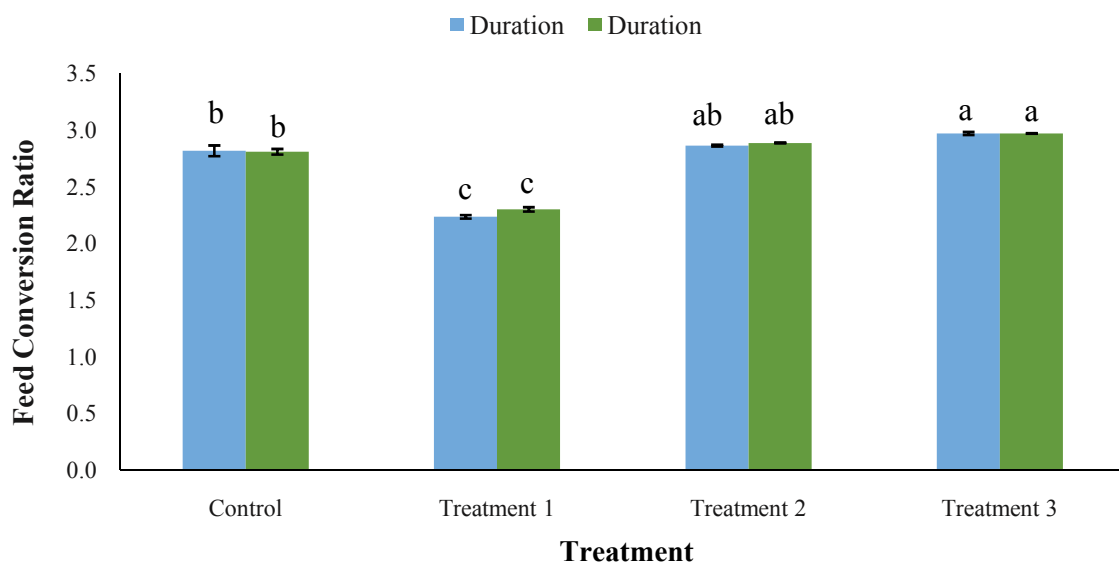
Figure 4:   The feed conversion ratio (FCR) comparison between 30 day and 60 day rearing period of the *Pangasius hypophthalmus* in different treatment method. Data are represented as Mean ± SEM. Bars with same color different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.1.5 Viscerosomatic Index (VSI %)

Viscerosomatic index (VSI) was calculated at the end of the experiment. Table 6 shows the values. The highest (8.985 $\pm$ 0.005) value of viscerosomatic index was observed from the Treatment-3 group where 5% enzymatically treated feed was given while the least (8.255 $\pm$ 0.025) was observed in the Treatment-1 group where 3% enzymatically treated feed was given.However the control treatment and Treatment-2 group showed a value of (8.610 $\pm$ 0.010) and (8.445 $\pm$ 0.025) respectively in case of viscerosomatic index.Again all the treatment group showed significant difference among them (Figure 5).

Figure 5: The viscerosomatic index (VSI) comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean $\pm$ SEM. Bars with different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.1.6 Hepatosomatic Index (HSI %)

Hepatosomatic index (HSI) was calculated at the end of the rearing period. Table 6 shows the values.The enzyme supplementation increased the HSI but the increased amount was not significant. Treatment-1 where 3% enzymatically treated feed was given showed the highest (2.405 $\pm$ 0.005) value of hepatosomatic index (HSI) while the control group where feed without enzyme was given showed the least (2.285 $\pm$ 0.015) value. Howeverthere was no significant difference between them while the other two treatment group showed similar pattern in the hepatosomatic index value (Figure 6).

Figure 6: The hepatosomatic index (HSI) comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean ± SEM.

3.1.7 Flesh Yield (FY %)

Flesh yield (FY) was calculated at the end of the experiment. Table 6 shows the values. The highest (48.560 $\pm$ 0.310) value of flesh yield was observed in the Treatment-1 group where 3% enzyme mixed feed was given. In contrast, the lowest (46.585 $\pm$ 0.045) value was observed in the Treatment-3 where 5% enzymatically treated feed was given. These two group showed significant difference between them. Moreover the control group showed another significantly difference value (47.630 $\pm$ 0.170) of flesh yield from the earlier two treatment while the increase dose of enzyme decreased the flesh yield gradually (Figure 7).

Figure 7: The flesh yield (FY %) comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean ± SEM. Bars with different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.2 Nutritional Quality

There are several parameters which could be observed as a nutritional quality indicator. In this study, moisture, ash, protein and lipid content were observed as an indicator of nutritional quality of the experimented fish. At the initial stage of the experiment, proximate composition of the fish body was done (Table 7) and at the end of the experiment the proximate composition was done once again (Table 8). These nutritional parameter were compared only with the final record of treatment tanks in this experiment to see the best effect of different diet feeding.

Table 7: Initial proximate composition of the experimental fish.

Parameters	Percentage (%)
Crude Protein	24.79
Crude Lipid	11.71
Ash	1.13
Moisture	62.14

Table8: Proximate composition of the experimental fish after 60 days rearing period.

Treatment	Replication	Moisture (%)	Ash (%)	Protein (%)	Lipid (%)	Total
Control	1	61.73	1.22	24.814	11.78	99.544
	2	62.42	1.18	24.497	11.62	99.717
Treatment - 1	1	59.39	1.24	26.371	12.14	99.141
	2	58.94	1.28	26.783	12.08	99.083
Treatment - 2	1	60.08	1.22	25.510	12.27	99.080
	2	60.59	1.20	25.577	12.02	99.387
Treatment - 3	1	60.63	1.31	25.403	12.45	99.793
	2	60.30	1.25	25.094	12.23	98.874

3.2.1 Moisture Content (%)

Moisture content was measured after 60 days rearing period (Table 8) where the control group showed the highest (62.075 $\pm$ 0.345) moisture content. In contrast, the Treatment-1 showed the least (59.165 $\pm$ 0.225) moisture content value. However the other two treatment group (60.335 $\pm$ 0.255 for Treatment-2 group and 60.465 $\pm$ 0.165 for Treatment-3 group) showed a similar pattern to the moisture content value like the Treatement-1 group. Again, there is no significant difference between three enzymatically treated treatment groups in moisture content while the control group showed a significant difference than any of the other treatment group (Figure 8).
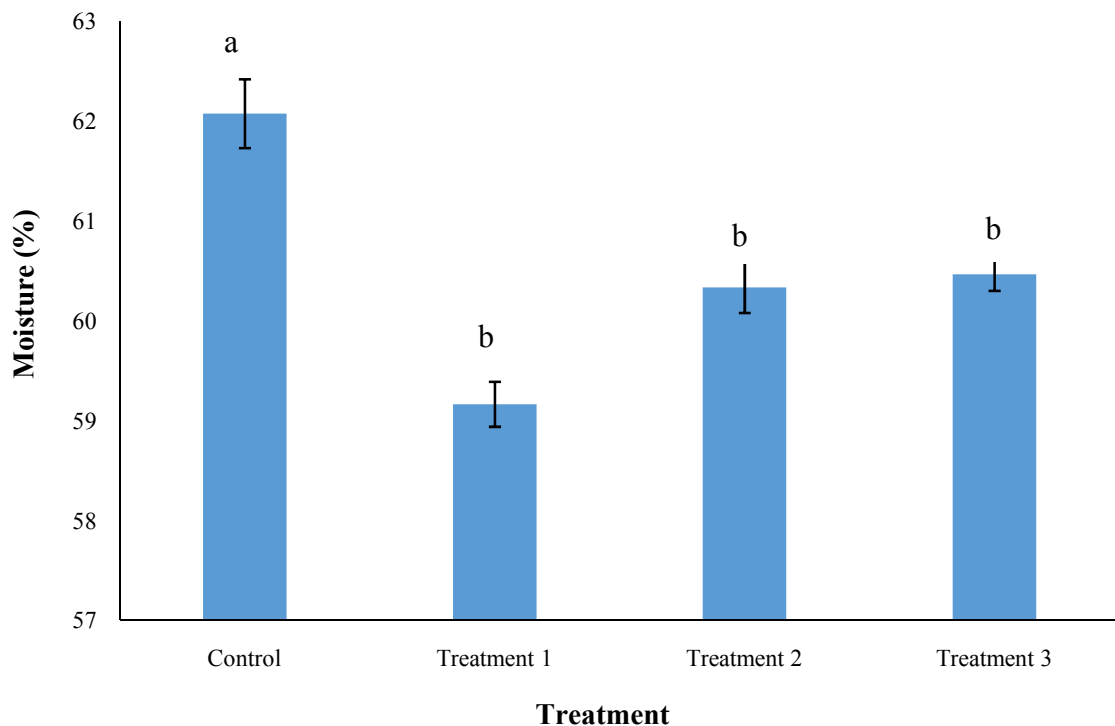
Figure 8: The moisture content comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean ± SEM. Bars with different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.2.2 Ash Content (%)

After 60 days of rearing period, the ash content was measured (Table 8). The highest (1.280 ± 0.030) value of ash content was observed in the Treatment-3 group where 5% enzyme mixed feed was given. On the other hand, the least (1.200 ± 0.020) valued was seen in the control group where enzymatically untreated feed was given. Again the other treatment group showed similar value of ash content; 1.260 ± 0.020 for Treatment-1 where 3% enzyme mixed feed was given and 1.210 ± 0.010 for Treatment-2 where 4% enzyme mixed feed was given. However there was no significant difference between any of the treatment group (Figure 9).
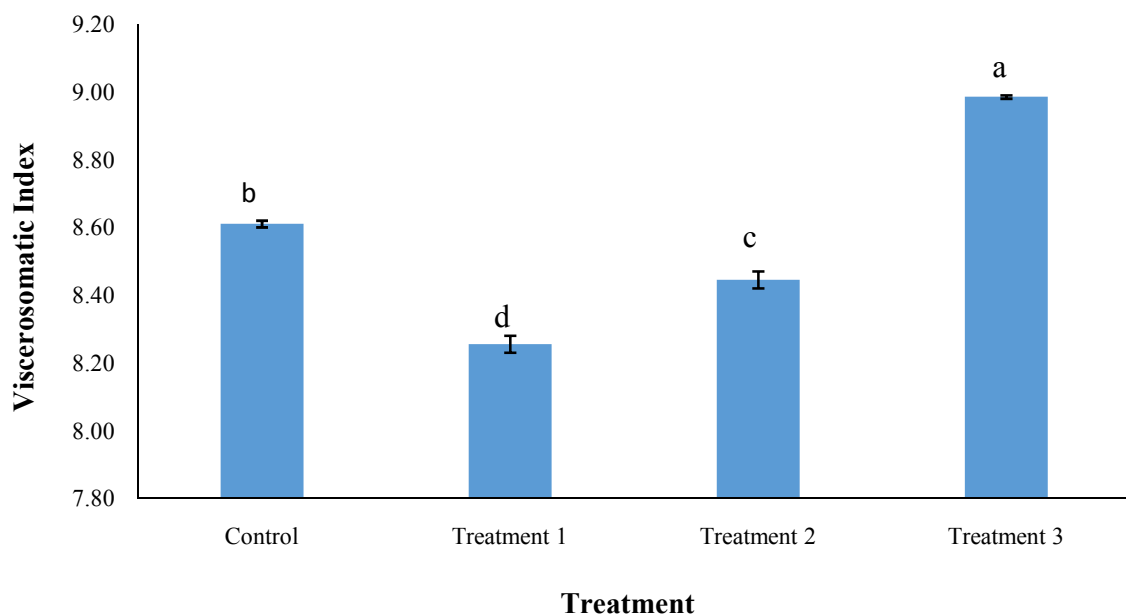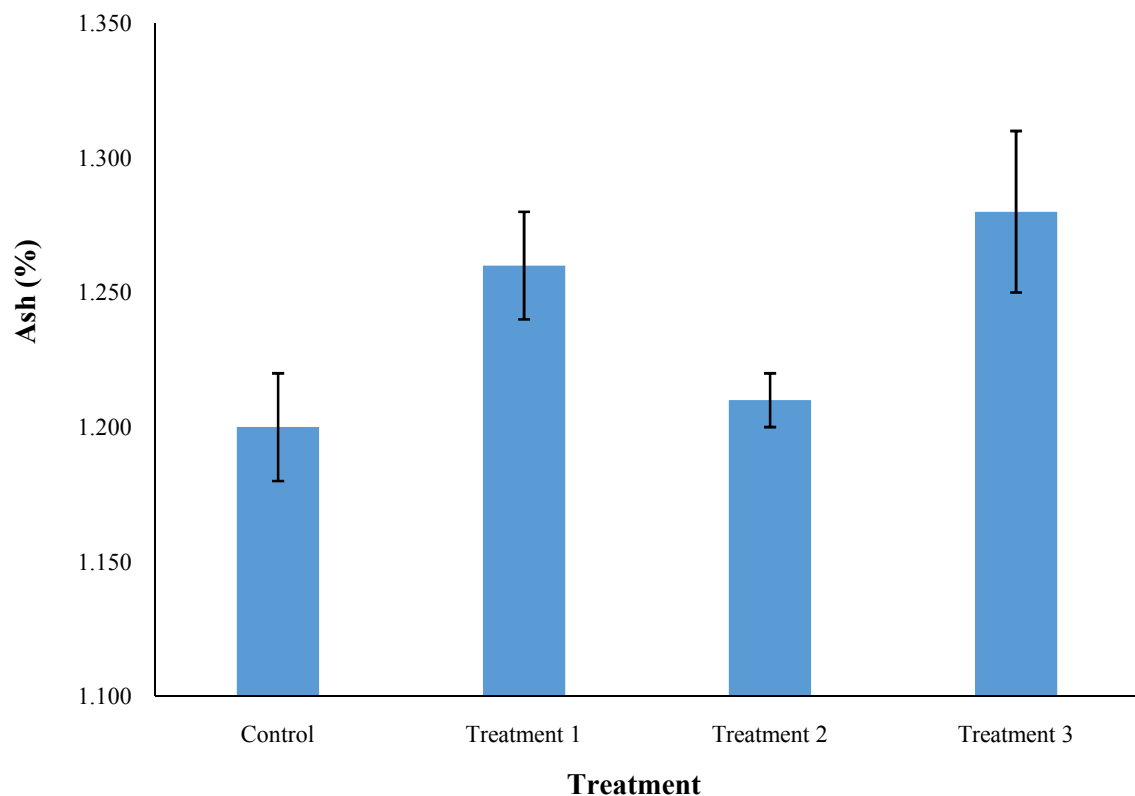

Figure 9: The ash content comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean ± SEM

3.3.3 Crude Protein Content (%)

After the end of the rearing period, the crude protein content was measured (Table 8). The best and highest (26.577 $\pm$ 0.206) crude protein content was found in the Treatment-1 group where 3% enzyme mixed feed was given. Again the least (24.544 $\pm$ 0.034) value of crude protein content was found in the Treatment-2 where 4% enzyme mixed feed was given. However the crude protein content of Treatment-1 was significantly different from the other treatments while the other two treatment showed similar pattern of crude protein content value (24.655 $\pm$ 0.159 for control group and 25.249 $\pm$ 0.155 for Treatment-3). Moreover there was significant difference between control group and Treatment-3 group in the crude protein content value while Treatment-3 showed an average value of protein content than control group and Treatment-2 group (Figure 10).
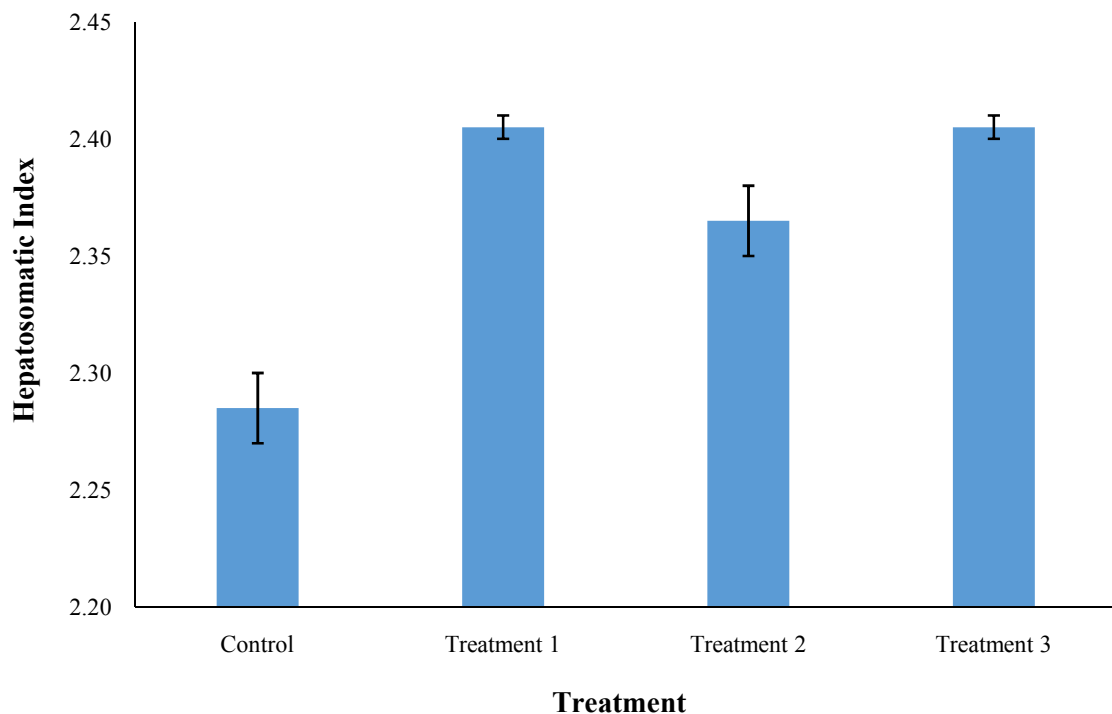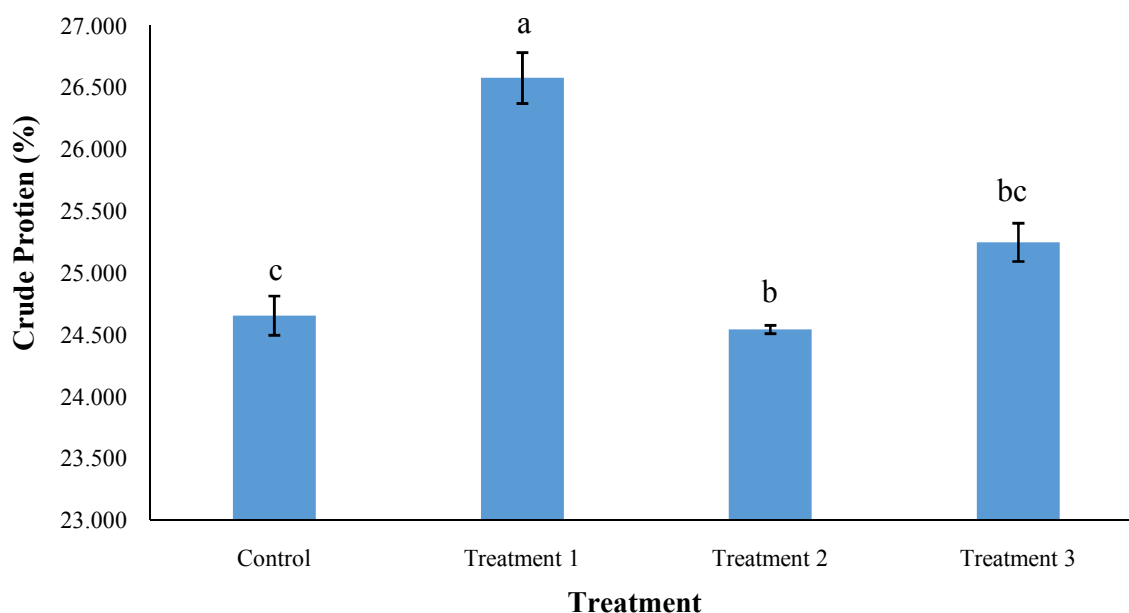

Figure 10: The crude protein content comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean $\pm$ SEM. Bars with different letters are significantly different from each other (p<0.05, Tukey, LSD).

3.3.4 Crude Lipid Content (%)

Crude lipid content was measured at the end of the experiment (Table 8). Treatment-3 showed the highest (12.340 ± 0.110) value of crude lipid content where 5% enzyme mixed feed was given. On the other hand, the least (11.700 ± 0.080) value of crude protein content was observed in the control group. This two treatment group showed significant difference between them. However the Treatment-1 group and Treatment-2 group showed a similar pattern in crude lipid content without any significant difference (Figure 11).
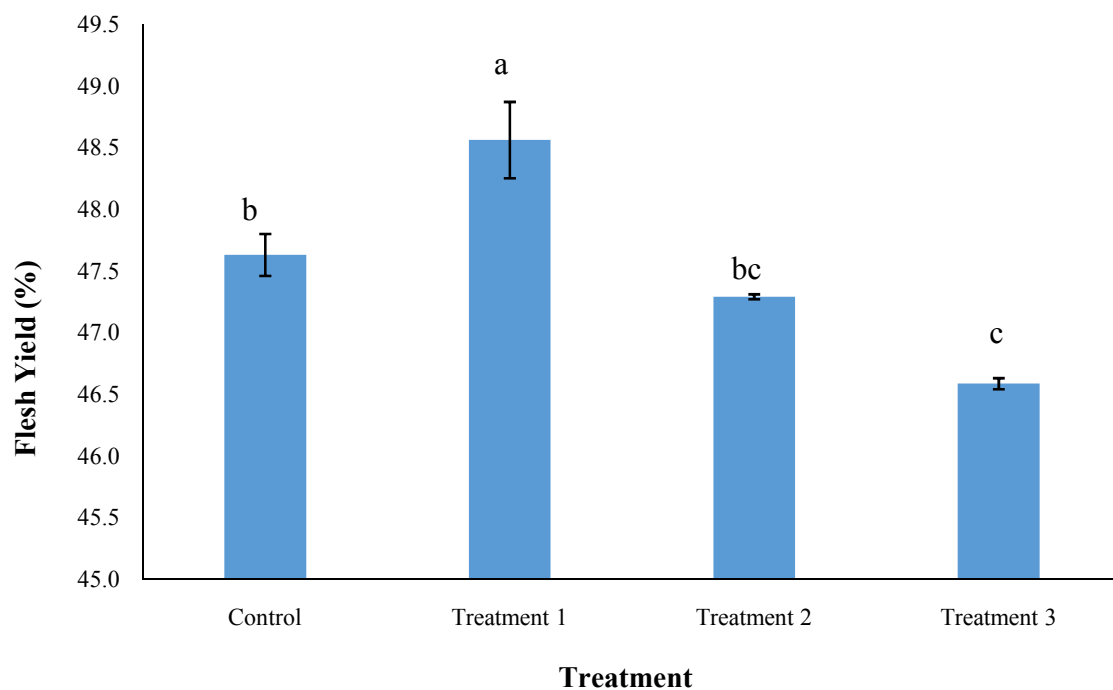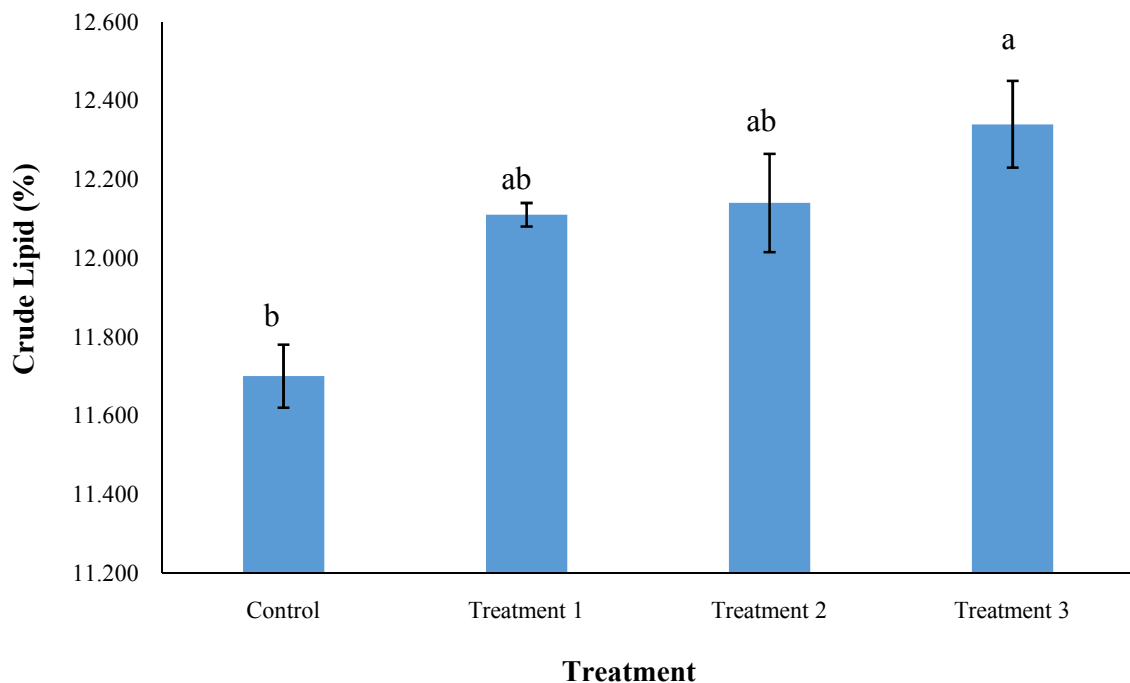

Figure 11: The crude lipid content comparison between different treatment methods of the *Pangasius hypophthalmus*. Data are represented as Mean ± SEM. Bars with different letters are significantly different from each other ($p < 0.05$, Tukey, LSD).

CHAPTER 4: DISCUSSION

4.1 Growth Performances

4.1.1 Condition Factor (k)

Each fish has a characteristic range of condition factors, which reflects their body conformation. It is an indicator of fish growth. In fisheries biology it is used to measure the variation from expected weight for length of individual fish or groups of individual.

The condition factor of *Pangasius hypophthalmus* was determined at the initial point of the study,after 30 days and after 60 days of the study. In this study, the highest ($0.876\pm$ 0.009) condition factor was found in Treatment-1 group where 3% enzyme mixed feed was supplemented. However this was significantly different from the other treatment group even from control group. The highest condition factor denotes that the fish under 3% enzymatically treated feed has obtained the best growth in proportion to others with a significant difference. But with the increase of the dose of enzyme supplementation, the FCR gone lower. The higher rate of enzyme supplementation may cause some negative activities on the feed efficiency of the fish resulting the lower FCR with higher does.

Geode and Barton (1990) reviewed that, declined condition factor was observed in fish suffer from stress. However the findings got similarities with those of Saha *et al.* (1998) who got this value of condition factor as nearer to 1 in case of *Clarias batrachus* fed on formulated diets. Bersa (1997) also observed condition factornearly 1 in *Anabas testudienus*. Rahman *et al.* (1997) showed in a study on the growth of catfish after giving selected supplemental feeds for the values of condition factor between 0.81-0.87 which coincides with this experiment result.

4.1.2 Average Daily Gain (ADG)

The average daily gain (ADG) was increased a bit with the enzyme supplementation at a lower rate. But with the higher dose of enzyme supplementation, the ADG decreased at a dose dependent manner. The highest (0.225 ± 0.020) average daily gain (ADG) was observed in the group fed with 3% enzyme mixed feed while the lowest (0.195 ± 0.040)

was observed in the group fed with 5% enzyme mixed feed. Although there was no significant difference between the treatments but the highest ADG refer to the better growth of the fish which was done under 3% enzyme supplemented population.

This difference in ADG could be due to better utilization of enzyme mixed feed. Appropriate level of enzyme might help for better growth by better assimilation of diet. Different fish species showed different ADG in different age. Sangrattanakhul (1989) found that the ADG of *Anabas testudineus* fish ranging from 0.10-0.12g in weight. Again Shahjahan (1997) found the ADG of GIFT tilapia ranging from 0.14-0.175g in weight. Higher ADG of 1.87 $\pm$ 0.21 also reported in a report of Ahmed *et al.* (2013).Different trends of result also found. Mandal *et al.*(2002) reported that the ADG differs significantly with the stocking density.However the ADG found in this study was higher than Sangrattanakhul's and Shahjahan's experiment but lower than Ahmed*et al.* experiment. But it denotes that the 3% enzyme mixed feed was much better than others.

4.1.3 Specific Growth Rate (SGR)

The highest (1.6237 $\pm$ 0.05) specific growth rate (SGR) was found in Treatment-1 group where 3% enzyme mixed supplemented feed was given, where the lowest (1.2621 $\pm$ 0.08) specific growth rate was found in Treatment-2. Although there was no significant differences in SGR between treatment groups but a significant decrease in rearing period was seen. The SGR of 3% enzyme mixed feed supplied population was 1.700 at the end of the 30 day rearing period while the SGR decreased to 1.620 at the end of the 60 day rearing period. Moreover the SGR decreased to 1.270 from1.370 for Treatment-2 group where 4% enzyme mixed feed was supplemented. However with the increase of age, there was a decrease in SGR which indicate the Medawar's (1945) fifth law; "Organism age fastest when they are young". Minot (1908) was the first person to recognize that for most animals, the specific growth rate is highest early in life and that it typically decreases with increasing age, becoming zero in some animals.

Yildrim *et al.* (2010) found that the significantly higher and best specific growth rate of African Catfish (*Clarius gariepinus)* was observed at the group receiving 0.75gKg[-1] enzyme complex group while the range of SGR was 1.09-1.23g in weight. Ahmed *et al.* (2013) found that the SGR of Thai Pangas were 1.13 $\pm$ 0.06. Mandal *et al.* (2002)

reported that the SGR of *Pangasius sutchi* fry ranges from 0.1268-0.2085. Again Essa *et al.* (2010) showed that the SGR can be from 0.84 to 1.38 in case of Nile Tilapia, *Oreochromis niloticus* with the application of dietary probiotics.Different trends of result also found. Mandal *et al.*(2002) reported that the SGR differs significantly with the stocking density. Discussing the other experiments, it can be said that the SGR value of 1.6237 in this experiment for the Treatment-1 group denotes that 3% enzyme supplementation helps to grow the *Pangasius hypophthalmus* more rapidly than other.

4.1.4 Feed Conversion Ratio (FCR)

Feed conversion ratio (FCR) values of *Pangasius hypophthalmus* fish fed with different feed was observed from the record that ranges from 2.236-2.971. However with the introduction of the enzyme supplementation in feed, the FCR decreased. Again with the increasing rate of the enzyme supplementation more than 3%, the FCR increased in a gradual manner. In this studythe lowest and best Feed Conversion Ratio (2.236± 0.019) was found in the treatment-1 where the fish were fed with 3% enzyme mixed feed and the highest (2.971 ± 0.003) FCR was found in the Treatment-3 groups where 5% enzyme mixed feed was given. The FCR of Treatment-1 was significantly different from the other treatment. It could be due to better utilization of feed.Also, supplementation the diets with mixture of multi enzyme improved growth performance, protein digestibility, and nutrients utilization of Nile tilapia (Zhong and Zhou, 2005). The maximum weight gain was obtained by feeding 3% enzyme mixed feed. But with the increase of enzyme percentage the FCR decreased. The lower the FCR, better the feed conversion to fish flesh. From this point of view the 3% enzymatically treated feed gives the best result in comparison with other enzymatically treated feed. The feed conversion ratio (FCR) in the experiment is ranging from 2.236-2.971which is almost same with the FCR value of *H. fossilis* founded Akand *et al.* (1989).

However the FCR was improved with the 3% enzyme supplementation. The improved feed conversion due to enzyme supplementation in the present study is supported by the findings of (Lund, 1987; Gadient and Broze, 1992; Vranjes *et al.*,1994, Jamroz *et al.*, 1995; Al Bustany, 1996; Wang *et al.*,1997 and Huazhong *et al.*,1999). Tahounl *et al.* (2011) found that, the multi enzyme complex has a positive effect on FCR of fish when supplemented with 0.5g/Kg.Ahmed *et al.* (2013) found that the feed conversion ration

(FCR) varies from 1.93 $\pm$ 0.30 and 2.34 $\pm$ 0.12 in *Pangasius hypophthalmus*. This statistics justify the current result of this study.Yildrim *et al.* (2010) found that the best specific feed conversion ratio of African Catfish (*Clarius gariepinus)* was observed at the group receiving 0.75gKg^{-1} other than the control group or other enzymatically treated treatment.

4.1.5 Viscerosomatic Index (VSI %)

After the 60 days rearing period the viscerosomatic index were measure for each treatment group. All the four different group showed significantly different VSI. Small amount of enzyme supplementation decreased the VSI but then with the increasing rate of enzyme supplementation, the VSI increased in a dose depended manner while the difference was significant from each other.The highest (8.985 $\pm$ 0.005) value of viscerosomatic index was observed in the group fed with 5% enzymatically treated feed while the least (8.255 $\pm$ 0.025) was observed in the group fed with 3% enzymatically treated feed. The enzyme supplement helped to increase the viscerosomatic index.

Phumee *et al.* (2009) reported that, there was no significant differences in viscerosomatic index while experimenting the effects of dietary protein and lipid content on growth performance and biological indices of *Pangasius hypophthalmus* fry where VSI varied from 5.84 to 7.79. In another study, Aliyu-Paiko *et al.* (2010) found similar VSI value ranging from 3.5 to 4.8 while experimenting the effects of dietary lipid/protein ratio on *Channa striatus*. Ighwela *et al*. (2014) found that there was no significant differences in viscerosomatic index in maltose supplemented feed compared to control treatment while the control group showed a higher viscerosomatic index than the maltose supplemented treatment.

4.1.6 Hepatosomatic Index (HSI %)

There was no significant difference in the hepatosomatic index between the treatment groups in this study. However the highest (2.405 $\pm$ 0.005) value was obtained from the 3% enzyme mixed supplemented population while the least (2.285 $\pm$ 0.015) value was obtained from the control group. May be the enzyme supplement had a smaller and insignificant effluence on the hepatosomatic index.

Phumee *et al.* (2009) found that, hepatosomatic index (HSI) was significantly affected by the level of protein while experimenting the effects of dietary protein and lipid content on growth performance and biological indices of *Pangasius hypophthalmus* fry where HSI varied from 1.70 to 2.09. Ighwela *et al.* (2014) found that there was a significant differences in hepatosomatic index in maltose supplemented feed compared to control treatment. In another study, Aliyu-Paiko *et al.* (2010) found similar HSI value ranging from 2.1 to 2.6 while experimenting the effects of dietary lipid/protein ratio on *Channa striatus*.

4.1.7 Flesh Yield (FY %)

The flesh yield was significantly higher (48.560 $\pm$ 0.310) in the Treatment-1 group where 3% enzyme mixed feed was supplemented. In contrast, the lowest (46.585 $\pm$ 0.045) value was observed in the Treatment-3 where 5% enzymatically treated feed was given. A higher value of flesh yield denotes the growth at a higher rate. However with the increasing rate of the enzyme dose, the flesh yield decreased consistently. May be higher rate of enzyme supplementation blocked the way of proper utilization of feed in those treatment.

4.2 Nutritional Quality

4.2.1 Moisture Content (%)

The enzyme content decreased the moisture content in this study. All the treatment which were given enzyme mixed feed showed a significant negative difference in moisture content than the control treatment.However the lowest moisture content was found in 3% enzyme mixed feed supplemented treatment. Again 4% and 5% enzyme mixed feed supplemented treatment showed a higher moisture content than the 3% enzyme mixed feed treated treatment but still lower than the control group.

Siti-Norita *et al.* (2015) found that the moisture content of tilapia fed with diets containing enzyme were positively correlated however there was no significant differences. Phumee *et al.* (2009) found that, body moisture content was not influenced by either protein or lipid levels in the diet. Dada and Olugbemi (2013) found the moisture content of *Clarias gariepinus* did not differ among treatments in addition of

feed additives. Essa *et al.* (2010) also found the Moisture content showed no significant differences among fish fed the experimental diets.Again, Eroldogan*el al.* (2004) reported a significant difference in moisture content with the supplementation. This sets of result coincide the result of the current study.

4.2.2 Ash Content (%)

There was no pattern in ash content within the treatment groups. However no significant difference was seen within them. The highest (1.280 $\pm$ 0.030) value of ash content was observed in the Treatment-3 group where 5% enzyme mixed feed was given. On the other hand, the least (1.200 $\pm$ 0.020) valued was seen in the control group where enzymatically untreated feed was given.

Dada and Olugbemi (2013) reported that the ash content was significantly affected by the feed additives in case of *Clarias gariepinus*. Siti-Norita *et al.* (2015) found that the β-Mannanase supplementation led to an increase in ash compared with the control.

4.2.3 Crude Protein Content (%)

As the other parameters, crude protein content was higher in the enzyme mixed feed supplemented treatment. The highest (26.577 $\pm$ 0.206) crude protein content was found in the Treatment-1 group where 3% enzyme mixed feed was given. This was significantly higher than the other treatments. However the crude protein content was higher in Treatment-3 group than the control while the Treatment-2 group showed a less crude protein content than the control group. As 3% enzyme mixed feed increased the crude protein level in a significant manner, then this composition of feed with enzyme is better for the nutritional improvement.

Dada and Olugbemi (2013) found that the highest protein content (67.15%) was obtained in the fish fed with dietary Aqua pro® and it was significantly higher than in all other groups in case of *Clarias gariepinus*. However no effect on protein content was also reported. Eroldogan *et al.* (2004) reported that, there were no significant differences in protein in European sea bass *Dicentrarchus labrax* after the feeding trial.

4.2.4 Crude Lipid Content (%)

A dose dependent increase in crude lipid content was found in this experiment while control group showed the least (11.700 $\pm$ 0.080) lipid content and Treatment-3 showed the highest (12.340 $\pm$ 0.110) value. The crude protein content of Treatment-3 group was significantly higher than the control group.

Eroldogan *et al.* (2004) reported that the lipid content relate with the feeding rate. Again, Dada and Olugbemi (2013) found that the lipid content is highly affected by feed additives. This reports coincide the current result.

CHAPTER 5: CONCLUSION

The present study showed the effects of enzyme supplementation on the growth performance and nutritional quality of Thai Pangas (*Pangasius hypophthalmus*) during rearing and feeding in the laboratory condition for 60 days. Three enzymatically treated feed were used in the feeding and rearing trial of the fish. Different growth parameters, such as condition factor, average daily gain, specific growth rate, feed conversion ratio, viscerosomatic index, hepatosomatic index and flesh yield were observed along with some nutritional parameters such as moisture, ash, lipid and protein content observation.

A positive effect of the enzyme supplementation on condition factor, average daily gain, specific growth rate, feed conversion ratio, viscerosomatic index, flesh yield, moisture content, and crude protein content was found for 3% enzyme mixed feed supplementation.

However the effect of enzyme at a rate of 3% was significant for the condition factor, specific growth rate, feed conversion ratio, viscerosomatic index, flesh yield, moisture content and crude protein content.

Only the ash content and lipid content showed an increase manner with the higher dose where 5% enzyme mixed feed supplementation showed the highestvalue of both content while control group showed the least.

There was no significant difference between any treatment group of enzyme supplementation on average daily gain, hepatosomatic index and ash content.

On the basis of statistical analysis it was observed that the 3% enzyme mixed supplemented feed was the most effective for the growth and nutritional quality improvements of Thai Pangas, *Pangasius hypophthalmus* fish.

CHAPTER 6: RECOMMENDATIONS

The effects of enzyme supplementation on the growth performance and nutritional quality was assessed during this current study. More studies should be conducted on enzyme supplementation in embryos, larvae and brood stock.

This study showed 3% enzyme supplementation is much better than other dose where the effects of 1% and 2% dose was not estimated. Now it is needed to evaluate the more specific dose of enzyme supplementation in a lower dose or even in a fraction dose.

Effects of enzyme on the reproductive performance of Thai Pangas, *Pangasius hypophthalmus* may be needed.

This current study was completed in laboratory condition, so further field studies are needed to estimate the real effect of enzyme supplementation.

It is also needed to see the adverse effect of enzyme supplementation on the environment and public health.

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APPENDICES

Plate 1: Addition of the enzyme supplementation to the feed with micro pipette.

Plate 2: Properly mixing of enzyme with feed by a stick

Plate 3: Establishment and arrangement of experimental tanks.

Plate 4: Releasing of previously grouped experimental fish in the tank.

Plate 5: Measuring of the feed in appropriate amount with electric balance.

Plate 6: Feeding the treatment group with enzyme mixed feed.

Plate 7: Cleaning of tanks with the brush after every week.

Plate 8: Measurement of length of fish with wooden measuring scale

Plate 9: Measurement of weight of fish with electric balance.

Plate 10: Crucibles are placed in the oven to determine the moisture content.

Plate 11: Crucibles are placed in the muffle furnace to determine the ash content

Plate 12: Kjeldhal flask distillation during the determination of protein content.

Plate 13: A view of lipid extractor machine during the determination of lipid.

Appendix 1: ANOVA Table ofall the findings after 60 days rearing period

		Sum of Squares	df	Mean Square	F	Sig.
ANOVA						
Condition factor	Between Groups	.024	3	.008	30.588	.003
	Within Groups	.001	4	.000		
	Total	.025	7			
Average daily gain	Between Groups	.001	3	.000	.465	.722
	Within Groups	.004	4	.001		
	Total	.005	7			
Specific growth rate	Between Groups	.135	3	.045	5.064	.076
	Within Groups	.036	4	.009		
	Total	.171	7			
Feed conversion ratio	Between Groups	.544	3	.181	366.163	.000
	Within Groups	.002	4	.000		
	Total	.546	7			
Viscerosomatic Index	Between Groups	.577	3	.192	279.873	.000
	Within Groups	.003	4	.001		
	Total	.580	7			
Hepatosomatic Index	Between Groups	.019	3	.006	25.600	.005
	Within Groups	.001	4	.000		
	Total	.020	7			
Fish Yield	Between Groups	4.042	3	1.347	21.145	.006
	Within Groups	.255	4	.064		
	Total	4.296	7			
Moisture	Between Groups	8.582	3	2.861	21.845	.006
	Within Groups	.524	4	.131		
	Total	9.106	7			
Ash	Between Groups	.009	3	.003	3.315	.139
	Within Groups	.004	4	.001		
	Total	.013	7			
Crude Protein	Between Groups	3.876	3	1.292	27.921	.004
	Within Groups	.185	4	.046		
	Total	4.061	7			
Crude Lipid	Between Groups	.434	3	.145	8.260	.034
	Within Groups	.070	4	.018		
	Total	.504	7			

Appendix 2: Homogenous subset of feed conversion ratio (FCR)

Feed conversion ratio

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	3% enzyme supplement 30 day	2	2.2359		
	3% enzyme supplement 60 day	2	2.3015		
	Control 60 day	2		2.8096	
	Control 30 day	2		2.8182	
	4% enzyme supplement 30 day	2		2.8651	2.8651
	4% enzyme supplement 60 day	2		2.8872	2.8872
	5% enzyme supplement 60 day	2			2.9706
	5% enzyme supplement 30 day	2			2.9717
	Sig.		.457	.296	.090

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 3: Homogenous subset of average daily gain (ADG)

Average daily gain

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	5% enzyme supplement 30 day	2	.1750
	control 30 day	2	.1750
	3% enzyme supplement 30 day	2	.1850
	5% enzyme supplement 60 day	2	.1950
	Control 60 day	2	.1950
	4% enzyme supplement 30 day	2	.2000
	4% enzyme supplement 60 day	2	.2000
	3% enzyme supplement 60 day	2	.2250
	Sig.		.675

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 4: Homogenous subset of specific growth rate (SGR)

Specific growth rate

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	4% enzyme supplement 60 day	2	1.2671	
	5% enzyme supplement 30 day	2	1.3545	1.3545
	5% enzyme supplement 60 day	2	1.3668	1.3668
	4% enzyme supplement 30 day	2	1.3776	1.3776
	control 60 day	2	1.4233	1.4233
	control 30 day	2	1.5643	1.5643
	3% enzyme supplement 60 day	2	1.6237	1.6237
	3% enzyme supplement 30 day	2		1.6968
	Sig.		.089	.106

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 5: Homogenous subset of condition factor (k)

Condition factor

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	5% enzyme supplement initial	2	.72950	
	Control initial	2	.73400	
	Control 60 day	2	.73750	
	4% enzyme supplement initial	2	.73950	
	5% enzyme supplement 60 day	2	.74650	
	4% enzyme supplement 60 day	2	.77900	
	3% enzyme supplement initial	2		.86100
	3% enzyme supplement 60 day	2		.87550
	Sig.		.287	.992

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 6: Homogenous subset of viscerosomatic index (VSI)

Viscerosomatic Index

	Treatment	N	Subset for alpha = 0.05			
			1	2	3	4
Tukey B[a]	3% enzyme supplement	2	8.2550			
	4% enzyme supplement	2		8.4450		
	Control Treatment	2			8.6100	
	5% enzyme supplement	2				8.9850

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 7: Homogenous subset of hepatosomatic index (HSI)

Hepatosomatic Index

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	Control Treatment	2	2.2850
	4% enzyme supplement	2	2.3650
	3% enzyme supplement	2	2.4050
	5% enzyme supplement	2	2.4050

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 8: Homogenous subset of flesh yield (FY)

Flesh Yield

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	5% enzyme supplement	2	46.5850		
	4% enzyme supplement	2	47.2900	47.2900	
	Control Treatment	2		47.6300	
	3% enzyme supplement	2			48.5600

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 9: Homogenous subset of moisture content

Moisture

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey B[a]	3% enzyme supplement	2	59.1650	
	4% enzyme supplement	2	60.3350	
	5% enzyme supplement	2	60.4650	
	Control Treatment	2		62.0750

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 10: Homogenous subset of ash content

Ash

	Treatment	N	Subset for alpha = 0.05
			1
Tukey B[a]	Control Treatment	2	1.2000
	4% enzyme supplement	2	1.2100
	3% enzyme supplement	2	1.2600
	5% enzyme supplement	2	1.2800

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 11: Homogenous subset of crude protein content

Crude Protein

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey B[a]	Control Treatment	2	24.65550		
	5% enzyme supplement	2	25.24850	25.24850	
	4% enzyme supplement	2		25.54350	
	3% enzyme supplement	2			26.57700

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 12: Homogenous subset of crude lipid content

Crude Lipid

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey B[a]	Control Treatment	2	11.7000	
	3% enzyme supplement	2	12.1100	12.1100
	4% enzyme supplement	2	12.1450	12.1450
	5% enzyme supplement	2		12.3400

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 2.000.

Appendix 13: Tukey's post Hoc for multiple comparisons.

Multiple Comparisons

Dependent Variable		(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
							Lower Bound	Upper Bound
Condition factor	LSD	3% enzyme supplement	4% enzyme supplement	.108950*	.016096	.002	.06426	.15364
			5% enzyme supplement	.130350*	.016096	.001	.08566	.17504
			Control Treatment	.132850*	.016096	.001	.08816	.17754
		4% enzyme supplement	3% enzyme supplement	-.108950*	.016096	.002	-.15364	-.06426
			5% enzyme supplement	.021400	.016096	.254	-.02329	.06609
			Control Treatment	.023900	.016096	.212	-.02079	.06859
		5% enzyme supplement	3% enzyme supplement	-.130350*	.016096	.001	-.17504	-.08566
			4% enzyme supplement	-.021400	.016096	.254	-.06609	.02329
			Control Treatment	.002500	.016096	.884	-.04219	.04719
		Control Treatment	3% enzyme supplement	-.132850*	.016096	.001	-.17754	-.08816
			4% enzyme supplement	-.023900	.016096	.212	-.06859	.02079
			5% enzyme supplement	-.002500	.016096	.884	-.04719	.04219
ADG	LSD	3% enzyme supplement	4% enzyme supplement	.02500	.02979	.449	-.0577	.1077
			5% enzyme supplement	.03000	.02979	.371	-.0527	.1127

			Control Treatment	.03000	.02979	.371	-.0527	.1127
		4% enzyme supplement	3% enzyme supplement	-.02500	.02979	.449	-.1077	.0577
			5% enzyme supplement	.00500	.02979	.875	-.0777	.0877
			Control Treatment	.00500	.02979	.875	-.0777	.0877
		5% enzyme supplement	3% enzyme supplement	-.03000	.02979	.371	-.1127	.0527
			4% enzyme supplement	-.00500	.02979	.875	-.0877	.0777
			Control Treatment	.00000	.02979	1.000	-.0827	.0827
		Control Treatment	3% enzyme supplement	-.03000	.02979	.371	-.1127	.0527
			4% enzyme supplement	-.00500	.02979	.875	-.0877	.0777
			5% enzyme supplement	.00000	.02979	1.000	-.0827	.0827
Specific growth rate	LSD	3% enzyme supplement	4% enzyme supplement	.35659*	.09441	.019	.0945	.6187
			5% enzyme supplement	.25691	.09441	.053	-.0052	.5190
			Control Treatment	.20035	.09441	.101	-.0618	.4625
		4% enzyme supplement	3% enzyme supplement	-.35659*	.09441	.019	-.6187	-.0945
			5% enzyme supplement	-.09968	.09441	.351	-.3618	.1625
			Control Treatment	-.15624	.09441	.173	-.4184	.1059
		5% enzyme supplement	3% enzyme supplement	-.25691	.09441	.053	-.5190	.0052
			4% enzyme supplement	.09968	.09441	.351	-.1625	.3618
			Control Treatment	-.05656	.09441	.581	-.3187	.2056
		Control Treatment	3% enzyme supplement	-.20035	.09441	.101	-.4625	.0618
			4% enzyme supplement	.15624	.09441	.173	-.1059	.4184
			5% enzyme supplement	.05656	.09441	.581	-.2056	.3187
FCR	LSD	3% enzyme supplement	4% enzyme supplement	-.58570*	.02225	.000	-.6475	-.5239
			5% enzyme supplement	-.66908*	.02225	.000	-.7309	-.6073
			Control Treatment	-.50816*	.02225	.000	-.5699	-.4464
		4%	3% enzyme	.58570*	.02225	.000	.5239	.6475

				Mean Difference	Std. Error	Sig.	Lower	Upper
		enzyme supplement	supplement					
			5% enzyme supplement	-.08338*	.02225	.020	-.1452	-.0216
			Control Treatment	.07754*	.02225	.025	.0158	.1393
		5% enzyme supplement	3% enzyme supplement	.66908*	.02225	.000	.6073	.7309
			4% enzyme supplement	.08338*	.02225	.020	.0216	.1452
			Control Treatment	.16092*	.02225	.002	.0991	.2227
		Control Treatment	3% enzyme supplement	.50816*	.02225	.000	.4464	.5699
			4% enzyme supplement	-.07754*	.02225	.025	-.1393	-.0158
			5% enzyme supplement	-.16092*	.02225	.002	-.2227	-.0991
Viscerosomatic Index	LSD	3% enzyme supplement	4% enzyme supplement	-.19000*	.02622	.002	-.2628	-.1172
			5% enzyme supplement	-.73000*	.02622	.000	-.8028	-.6572
			Control Treatment	-.35500*	.02622	.000	-.4278	-.2822
		4% enzyme supplement	3% enzyme supplement	.19000*	.02622	.002	.1172	.2628
			5% enzyme supplement	-.54000*	.02622	.000	-.6128	-.4672
			Control Treatment	-.16500*	.02622	.003	-.2378	-.0922
		5% enzyme supplement	3% enzyme supplement	.73000*	.02622	.000	.6572	.8028
			4% enzyme supplement	.54000*	.02622	.000	.4672	.6128
			Control Treatment	.37500*	.02622	.000	.3022	.4478
		Control Treatment	3% enzyme supplement	.35500*	.02622	.000	.2822	.4278
			4% enzyme supplement	.16500*	.02622	.003	.0922	.2378
			5% enzyme supplement	-.37500*	.02622	.000	-.4478	-.3022
HSI	LSD	3% enzyme supplement	4% enzyme supplement	.04000	.01581	.065	-.0039	.0839
			5% enzyme supplement	.00000	.01581	1.00	-.0439	.0439
			Control Treatment	.12000	.01581	.002	.0761	.1639
		4% enzyme supplement	3% enzyme supplement	-.04000	.01581	.065	-.0839	.0039
			5% enzyme supplement	-.04000	.01581	.065	-.0839	.0039

			Control Treatment	.08000	.01581	.007	.0361	.1239
		5% enzyme supplement	3% enzyme supplement	.00000	.01581	1.00	-.0439	.0439
			4% enzyme supplement	.04000	.01581	.065	-.0039	.0839
			Control Treatment	.12000	.01581	.002	.0761	.1639
		Control Treatment	3% enzyme supplement	-.12000	.01581	.002	-.1639	-.0761
			4% enzyme supplement	-.08000	.01581	.007	-.1239	-.0361
			5% enzyme supplement	-.12000	.01581	.002	-.1639	-.0761
Fish Yield	LSD	3% enzyme supplement	4% enzyme supplement	1.27000*	.25241	.007	.5692	1.9708
			5% enzyme supplement	1.97500*	.25241	.001	1.2742	2.6758
			Control Treatment	.93000*	.25241	.021	.2292	1.6308
		4% enzyme supplement	3% enzyme supplement	-1.27000*	.25241	.007	-1.9708	-.5692
			5% enzyme supplement	.70500*	.25241	.049	.0042	1.4058
			Control Treatment	-.34000	.25241	.249	-1.0408	.3608
		5% enzyme supplement	3% enzyme supplement	-1.97500*	.25241	.001	-2.6758	-1.2742
			4% enzyme supplement	-.70500*	.25241	.049	-1.4058	-.0042
			Control Treatment	-1.04500*	.25241	.014	-1.7458	-.3442
		Control Treatment	3% enzyme supplement	-.93000*	.25241	.021	-1.6308	-.2292
			4% enzyme supplement	.34000	.25241	.249	-.3608	1.0408
			5% enzyme supplement	1.04500*	.25241	.014	.3442	1.7458
Moisture	LSD	3% enzyme supplement	4% enzyme supplement	-1.17000*	.36187	.032	-2.1747	-.1653
			5% enzyme supplement	-1.30000*	.36187	.023	-2.3047	-.2953
			Control Treatment	-2.91000*	.36187	.001	-3.9147	-1.9053
		4% enzyme supplement	3% enzyme supplement	1.17000*	.36187	.032	.1653	2.1747
			5% enzyme supplement	-.13000	.36187	.738	-1.1347	.8747
			Control Treatment	-1.74000*	.36187	.009	-2.7447	-.7353
		5%	3% enzyme	1.30000*	.36187	.023	.2953	2.3047

		enzyme supplement	supplement				
			4% enzyme supplement	.13000	.36187	.738	-.8747	1.1347
			Control Treatment	-1.61000*	.36187	.011	-2.6147	-.6053
		Control Treatment	3% enzyme supplement	2.91000*	.36187	.001	1.9053	3.9147
			4% enzyme supplement	1.74000*	.36187	.009	.7353	2.7447
			5% enzyme supplement	1.61000*	.36187	.011	.6053	2.6147
Ash	LSD	3% enzyme supplement	4% enzyme supplement	.05000	.03000	.171	-.0333	.1333
			5% enzyme supplement	-.02000	.03000	.541	-.1033	.0633
			Control Treatment	.06000	.03000	.116	-.0233	.1433
		4% enzyme supplement	3% enzyme supplement	-.05000	.03000	.171	-.1333	.0333
			5% enzyme supplement	-.07000	.03000	.080	-.1533	.0133
			Control Treatment	.01000	.03000	.756	-.0733	.0933
		5% enzyme supplement	3% enzyme supplement	.02000	.03000	.541	-.0633	.1033
			4% enzyme supplement	.07000	.03000	.080	-.0133	.1533
			Control Treatment	.08000	.03000	.056	-.0033	.1633
		Control Treatment	3% enzyme supplement	-.06000	.03000	.116	-.1433	.0233
			4% enzyme supplement	-.01000	.03000	.756	-.0933	.0733
			5% enzyme supplement	-.08000	.03000	.056	-.1633	.0033
Crude Protein	LSD	3% enzyme supplement	4% enzyme supplement	1.033500*	.215117	.009	.43624	1.63076
			5% enzyme supplement	1.328500*	.215117	.003	.73124	1.92576
			Control Treatment	1.921500*	.215117	.001	1.32424	2.51876
		4% enzyme supplement	3% enzyme supplement	-1.033500*	.215117	.009	-1.63076	-.43624
			5% enzyme supplement	.295000	.215117	.242	-.30226	.89226
			Control Treatment	.888000*	.215117	.015	.29074	1.48526
		5% enzyme supplement	3% enzyme supplement	-1.328500*	.215117	.003	-1.92576	-.73124
			4% enzyme supplement	-.295000	.215117	.242	-.89226	.30226

			Control Treatment	.593000	.215117	.051	-.00426	1.190266
		Control Treatment	3% enzyme supplement	-1.921500*	.215117	.001	-2.51876	-1.3242
			4% enzyme supplement	-.888000*	.215117	.015	-1.48526	-.29074
			5% enzyme supplement	-.593000	.215117	.051	-1.19026	.00426
Crude Lipid	LSD	3% enzyme supplement	4% enzyme supplement	-.03500	.13233	.804	-.4024	.3324
			5% enzyme supplement	-.23000	.13233	.157	-.5974	.1374
			Control Treatment	.41000*	.13233	.036	.0426	.7774
		4% enzyme supplement	3% enzyme supplement	.03500	.13233	.804	-.3324	.4024
			5% enzyme supplement	-.19500	.13233	.215	-.5624	.1724
			Control Treatment	.44500*	.13233	.028	.0776	.8124
		5% enzyme supplement	3% enzyme supplement	.23000	.13233	.157	-.1374	.5974
			4% enzyme supplement	.19500	.13233	.215	-.1724	.5624
			Control Treatment	.64000*	.13233	.008	.2726	1.0074
		Control Treatment	3% enzyme supplement	-.41000*	.13233	.036	-.7774	-.0426
			4% enzyme supplement	-.44500*	.13233	.028	-.8124	-.0776
			5% enzyme supplement	-.64000*	.13233	.008	-1.0074	-.2726
*. The mean difference is significant at the 0.05 level.								