CHAPTER-1

INTRODUCTION

Bangladesh has the third largest aquatic fish biodiversity in Asia, after China and India, with about 800 species in fresh, brackish and marine waters (Hussain and Mazid, 2001). This species diversity has been attributed to the world's largest flooded wetland (Bengal Delta) and the three main river systems (Ganges-Padma, Brahmaputra-Jamuna and Meghna) that flow from the Himalayas into the Bay of Bengal. This contributes to a high potential for fresh and brackish water capture and culture fisheries, in addition to the vast marine resources.

The favorable geographic position of Bangladesh comes with a large number of aquatic species and provides plenty of resources to support fisheries potential. Fish is the second most valuable agricultural crop in Bangladesh and its production contributes to the livelihoods and employment of millions of people. The culture and consumption of fish therefore has important implications for national income and food security. Fish is a popular complement to rice in the national diet, giving rise to the adage Maache-Bhate-Bangali ("a Bengali is made of fish and rice") (Ghose, 2014).

Bangladesh has achieved self-sufficiency in fish production. Bangladesh is ranked 5th in world aquaculture production (FAO, 2016). Bangladesh has recorded surplus fish production with an annual output of 41.34 lakh MT against a demand of 40.50 lakh MT (DoF, 2016). The fisheries sector plays a very important role in the national economy, contributing 3.65% to the Gross Domestic Product (GDP) of the country and 23.81% to the agricultural GDP (FRSS, 2017). Fish supplements about 60% of Bangladeshi people's daily animal protein intake (DoF, 2016). More than 17 million people including about 1.4 million women depend on fisheries sector for their livelihoods through fishing, farming, fish handling, and processing (BFTI, 2016). Fisheries and aquaculture sector have emerged as the second most important contributors to the export earnings of Bangladesh (Ghose, 2014). It is the second largest export industry in Bangladesh and produces 2.5% of the global production of shrimp.

Bangladesh is considered one of the most suitable countries in the world for freshwater aquaculture, because of its favourable resources and agro-climatic conditions. Overall, aquaculture plays an important role in the economy of Bangladesh, providing food, nutrition, incomes, livelihoods and export earnings (ADB, 2005a; Dey et al., 2010). Aquaculture is also considered to have the potential of food security in Bangladesh (Jahan et al., 2010). The culture of exotic carp, also commonly known as Chinese carp, including bighead carp, common carp, grass carp and silver carp has also been practiced in Bangladesh (ADB, 2005a). Other exotic species, such as tilapia and pangas has become popular in Bangladesh over the last decade.

The pangas aquaculture in Bangladesh has been emerged with an exotic species *Pangasius hypophthalmus* (Sauvage, 1878), also known as 'Pangas' or 'Thai Pangas'. *P. hypophthalmus* is one of the major fish species and one of the most important inland fisheries in the world. Among other cultured species *Pangasius* is the 4th most commonly cultured species in the world after salmon, shrimps and tilapia (Hekimoglu, 2014). This exotic species was brought from Thailand in 1989 and has been established as a cultured species in Bangladesh. Pangas production in pond systems was estimated at 156,375 tonnes in 2010-11 which was about 13% of total pond production (FRSS, 2012). This rapid growth has occurred due to its popularity to the pond farmers for possessing hardy characteristics, higher survival rates, fast growth, and ability to survive at high stocking densities. *Pangasius* also gain popularity among consumers due to its low market value, making it one of the most important cultured species, particularly among the poor in urban areas. The tremendous potential of the *Pangasius* sector directs the attention of world fisheries market.

Plate-1: *Pangasius hypophthalmus*

P. hypophthalmus mainly fed artificial feed. Feed cost is the major expense in pangus fish culture. One of the challenges is to develop less wasteful and more economic diets. Commercial fish feeds usually contain high fish meal content ranging from 30 to 50 %, but because of high cost and its scarcity, this component is generally omitted in the feed regions (Goda et al., 2007; Davies and Gouveia, 2008). Hence, aquaculture nutrition has been trying to improve the nutritional value of fish feed by enzyme supplementation in the last decades. Exogenous enzymes are now extensively used throughout the world as additives in animal diets. However, the effects of exogenous enzymes can be variable and are dependent on a large number of factors such as the age of animal and the quality and type of diet (Bedford and Schulze, 1998; Acamovic, 2001). Supplementation with enzymes is effective to eliminate the anti-nutritional factors and improve the utilization of dietary energy and amino acids, resulting in improved fish performance (Farhangi and Carter, 2007; Lin et al., 2007; Soltan, 2009). Supplementing fish feed with an enzyme or enzyme mixture that possess a broad-spectrum range of activities may improve the digestibility and as a result, growth performance in several cultured fish species like channel catfish (Jackson et al., 1996), *Pangasius pangasius* (Debnath et al., 2005), *Clarias batrachus* and *Clarias gariepinus* (Giri et al., 2003), tilapia (Drew et al., 2005) and salmon (Refstie et al., 1999; Odetallah et al., 2005). The digestibility of all nutrients, however including carbohydrates, protein, and minerals seems to be affected by exoenzymes (Felix and Selvaraj, 2004).

Although proteases produced naturally in the digestive system of fish, the addition of a specific exogenous protease could improve the protein digestibility. Nowadays, exogenous enzymes are extensively used all over the world as additives in fish diets to improve the nutritional value of fish feeds, especially with the raise of using plant proteins in aqua feeds and reduce water pollution (Kolkovski et al., 1997). Therefore, the present study was designed to investigate the establishment of the benefits of dietary enzyme supplementation for fish.

1.1 Aim and objectives of research:

The aim of this study is to describe the effects of pepsin enzyme on *P. hypophthalmus.*

The specific objectives of this research are to:

- To investigate the effects of commercially prepared diets supplemented with exogenous pepsin enzyme on the growth performance and feed utilization in *P. hypophthalmus*
- To determine the hematological profile of *P. hypophthalmus* feed with exogenous pepsin supplemented feed.

CHAPTER-2

REVIEW OF LITERATURE

For better understanding about the effects of exogenous protease enzyme (pepsin) on the growth performance, feed utilization and hematology parameters of fish it is essential to know the information about the previous related work. Addition of enzymes in the feed of different animals has been practiced to enhance digestive processes (Yildirim and Turan, 2010), however, not much work has been done in fish due to aquatic media. Available literature that shows that addition of digestive enzymes in artificial feed like other animals can enhance the digestive processes in fish (Sunde et al., 2004). The purpose of this chapter is to review the past studies conducted by different researchers to the related field.

Khalil et al. (2018) conducted an experiment on growth responses of striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) to exogenous enzyme added feed. At the end of feeding trial, growth rate was significantly increased in fish fed with enzyme supplemented diets in comparison with control group. The maximum increase in growth rate was recorded in 0.50 g kg^{-1} of α-amylase. Highest protein contents 68.18% was observed in 0.75g kg-1 α-amylase. Specific growth was higher in all enzyme supplemented groups as compared to control group. Hematological parameters were recorded among all groups and concentration of red blood cells (RBCs), white blood cells (WBCs), hematocrit (HCT) and numbers of lymphocytes were highest in treatment1 with the mean values of 1.29×10ˆ12 L^{-1}, 54.9×10ˆ9 L^{-1}, 18% and 48.7×10ˆ9 L^{-1}. They suggested that the enzyme supplementation improved the growth and health of *P. hypophthalmus*.

Lin et al. (2007) conducted an experiment on effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* × *O. aureus*. A commercial enzyme complex (neutral protease, b-glucanase and xylanase) was included at the level of 0.0 (control group), 1.0 and 1.5g kg $^{-1}$ diet in three test diets. Specific growth rate (SGR) and feed efficiency ratio (FER) showed increasing trends with increasing dietary enzyme level from 0.0 to 1.5g kg^{-1}. Fish fed the 1.5g kg^{-1} diet had the highest SGR (2.25% day^{-1}) and FER (64.5%). The results suggested that enzyme supplementation can significantly improve growth performance and feed utilization in juvenile hybrid tilapia.

Kolkovski et al. (1993) carried out an experiment on the effect of dietary exogenous digestive enzymes on ingestion, assimilation, growth and survival of gilthead seabream (*Sparus aurata*, Sparidae, Linnaeus) larvae. The results of the study suggested that assimilation rate was about 30% in larvae fed the microdiet with pancreatin as compared to the control diet. Larval growth over ten days was 0, 100 and 200% on microdiet free of added enzymes, one with added enzymes and a live food regime, respectively.

Zamini et al. (2014) carried an experiment on effects of two dietary exogenous multi-enzyme supplementation, Natuzyme® and beta-mannanase (Hemicell®), on growth and blood parameters of Caspian salmon (*Salmo trutta caspius*). After the ending of the experiment growth rate was found significantly higher in the exogenous enzyme group (1.01 ± 0.01) than the other groups. The best FCR (0.64 ± 0.015) was in the enzyme group and it was significantly lower than control (0.74 ± 0.02). SGR in the enzyme group (1.01 ± 0.01) was significantly higher than in the other groups. They also observed significant difference with respect to percentage body weight gain. No difference was observed in haematocrit%, Hb, RBC, MCV, MCH% or MCHC%. Monocyte count, Total WBC count, Total WBC count were significantly higher than control.

Lanari et al. (1998), reviewed by Kumar et al., (2012) suggested that addition of exogenous phytase in fish diets resulted in increased utilization of phytate phosphorous, other trace elements and protein and decreased discharge of phospshorous into the water.

Drew et al. (2005) conducted an experiment on effect of adding protease to coextruded flax:pea or canola: pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). They observed that the fish fed diets containing Coextruded flax : pea (F:P) or canola : pea (C:P) had reduced weight gain compared to the controls but there were no significant differences in specific growth rates. The addition of protease to the C:P diet resulted in a significant improvement in feed efficiency. However, the addition of protease to the F:P diet had no effect on growth.

Castillo and Gatlin (2015) suggested that supplementation of exogenous carbohydrases to plant-based fish diets should improve nutrient digestibility and reduce nutrient excretion. On the other hand, the effects of exogenous carbohydrases on fish performance are still unclear due to the difficulty in cross-study comparisons. Overall, based on the information gathered in this review, it is clear that research on exogenous carbohydrase supplementation in aquaculture nutrition is not extensive.

Dabrowska et al. (1979) carried an experiment on artificial diets for Common Carp: effect of the addition of enzyme extracts. The experiment showed only small positive effects of the addition of proteolytic enzymes in common carp diets. Enzyme was extracted from fish hepatopancreas and intestine. Then added to the diet processed by freeze drying. Survival and growth rate of the larvae were better but not as good as when the larvae were fed natural food.

Tahounl et al. (2011) conducted an experiment to investigate the effect of exogenous enzyme supplementation on reproductive performance of broodstock Nile tilapia reared in a hapa in pond hatchery system. They observed that the final body weight of male and female broodstock improved with the supplementation of exogenous enzyme and/or inclusion of 5% fish meal. They suggested that supplementation of enzyme and fish meal improved reproductive parameters value significantly.

Adeoye et al. (2016) studied on supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes. Tilapia fed the phytase supplemented diet displayed higher final body weight, FBW (94.9 g $fish^{-1}$) and specific growth rate, SGR (2.48% day^{-1}) compared to tilapia fed the control diet (82.6 g $fish^{-1}$ FBW and 2.11% day^{-1} SGR. In terms of feed conversion ratio, FCR and protein efficiency ratio, PER, tilapia fed diet supplemented with phytase (1.36 FCR and 1.08 PER) performed better than tilapia fed the control diet (1.68 FCR and 0.80 PER. The level of circulatory red blood cells was higher in tilapia fed the carbohydrase supplemented diet (1.98×10^6 μL^{-1}) compared to those fed the control diet. They found that the supplementation of diets with phytase has the potential to enhance tilapia growth without detrimental impacts on intestinal health.

Naela et al. (2017) studied on effect of a serine-protease on performance parameters and protein digestibility of cultured *Oreochromis niloticus* fed diets with different protein levels. The weight gain (WG), specific growth ratio (SGR) and protein

efficiency ratio (PER), apparent protein digestibility (APD) were significantly increased when 0 and 200 mg kg^{-1} diets were supplemented with protease enzyme while feed conversion ratio (FCR) was significantly decreased. Fish fed 200 mg kg^{-1} diet supplemented with 400 mg kg^{-1} protease showed a non-significant difference in final weight, SGR, PER and APD comparing with fish fed 0 mg kg^{-1} diet only. They suggested that the protease enzyme supplementation can significantly improve growth performance and protein digestibility in *O. niloticus*. Protease enzyme could be used to reduce the protein content of the diet with maintaining the fish performance.

Singh et al. (2011) carried an experiment on exogenous supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*. The effect of different levels of papain (1%, 2% and 4%) supplemented feed in *Cyprinus carpio* on growth rate, nutrient digestibility, gross protein retention (GPR), feed conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein utilization (ANPU), energy conversion efficiency (ECE%) and nitrogen retention efficiency (NRE) was studied. The fish fed on the feed supplemented with 2% papain treatment resulted in lowest feed conversion ratio (FCR), better growth rate, high protein digestibility, higher protein efficiency ratio (PER), good gross energy retention, better apparent net protein utilization (ANPU), energy conversion efficiency ECE (%) and nitrogen retention efficiency (NRE). All these parameters were lowest in case of control.

Yildirim and Turan (2010) studied on effects of exogenous enzyme supplementation in diets on growth and feed utilization in African Catfish, *Clarias gariepinus*. A multi enzyme complex (containing fungal xylanase, β-glucanase, pentosonase, β-amilase, fungal β-glucanase, hemicellulase, pectinase, cellulase, cellubiase), was included at the level of 0.0 (control group), 0.25, 0.5 and 0.75 g enzyme complex kg^{-1} diet in four test diets. The best specific growth rate was observed at the group receiving 0.75 g kg^{1} enzyme complex group. Also, food conversion ratio, protein efficiency ratio and apparent net protein utilization were significantly higher in all enzyme complex groups than that with control. The highest value of protein content (21.75%) was observed at 0.75 g kg^{-1} enzyme complex group. They suggested that enzyme supplementation can significantly improve growth performance and feed utilization in African catfish.

All of the studies indicated that enzymes additive feed exhibited beneficial effect on growth performance and feed utilization in *P. hypophthalmus*. On the other hand, enzyme supplementation also effects on blood parameters. Keeping in view all these aspects, the present study was conducted to evaluate the effect of different dosses of the pepsin enzyme on the growth performance, feed utilization and hematological parameters of *P. hypophthalmus* considering that pepsin enzyme will improve all these parameters.

CHAPTER-3

MATERIALS AND METHODS

This chapter deals with the methods that are followed and materials used to observe the effects of different dietary levels of pepsin on the growth performance, feed utilization and hematology parameters in *P. hypophthalmus*. In this experiment fishes were reared and maintained in the recirculatory system for 3 months by providing different dietary levels of pepsin in feed to observe the growth, feed utilization and hematology parameters of *P. hypophthalmus.*

3.1. Description of the study area

The experiment was conducted in aquarium with proper recirculation facilities at the Wet Laboratory of the Faculty of Fisheries, Chittagong Veterinary and Animal Sciences University, Chittagong for a period of 3 months from March to May, 2018.

3.2. Description of experimental system

The experimental unit consists of 15 rectangular glass aquaria (size 60 x 30 x 45 cm) containing about 70 liter of water. There had another two big sized glass aquaria for conditioning and stocking of fish. All the aquaria were placed in a metal frame for easy handling which facilitated better observation and accessibility. Underground water was used in the aquaria during experimental period. The water was continuously aerated by an aerator to maintain adequate level of oxygen in each aquarium.

3.3. Preparation of recirculatory system

The entire recirculatory system consisted of two identical unit. Each unit had 8 glass aquaria. Water was supplied from 250 L water tank by 1 HP water pump (Model: RSJ 10M, RFL) through recirculatory pipe. That recirculatory water pipe was placed up to the bottom of each aquarium so that it can siphon sedimented waste particles along with the suspended particles of the aquarium to the biological filter.

Freshwater was added to the recirculatory system from tap when needed to fill up the loss due to evaporation. About 10% of the total water in the system was flashed out and replenished with tap water daily. The biological filter drums contained filter sponge that promoted settling of wastes by increasing retention time and provided a substrate for attachment of nitrifying bacteria. Two different sizes and a layer of gravel stones along with a layer of charcoal were used in the biological filter for the filtration of water. Charcoal can remove dissolved organic molecules, chemicals, chlorine and chloramines and certain heavy metals.

Plate-2. Setting up aquarium and recirculatory system

3.4. Collection of fish and conditioning

Three hundred juvenile *P. hypophthalmus* fish with average initial weight 30.3±0.1g were collected from local Fisheries Hatchery, Chittagong. Prior to the start of experiment, fish were acclimatized for 10 days in storage aquarium prior to the experiment. During conditioning sufficient oxygen supply was maintained through artificial aeration. During the acclimation and experimental trail (90 days) fishes were feed twice daily (at around 9.00 a.m. and 5.00 p.m.) with a feeding rate of 7% of the total body weight basis.

Plate-3. Conditioning of fish

3.5. Experimental design

Five different levels of Pepsin viz. 0 g (control), 0.25 g, 0.50g, 0.75g and 1g pepsin/kg feed were administered as treatment T1, T2, T3, T4, and T5 respectively. Each treatment has three replications as R1 R2 and R3 and 20 fishes per replication representing 5 nutritional groups. These 15 aquariums divided into five groups and it corresponds to five experimental treatments.

3.6. Stocking of fish

After weighing individually, 20 fish per tank were randomly distributed and released into 15 tanks according to the experimental design. Continuous aeration was provided during the investigation period.

Plate-4. Weighing of fish before stocking

Plate-5. Fish stocked in aquarium

3.7. Cleaning of aquarium

The left over feed particles, faeces and debris were removed by siphoning method regularly. At the time of sampling the aquarium walls and bottoms were rubbed gently with sponge to remove the adhesive contents. After that treated with aquarium salt and washed thoroughly. Then the whole recirculatory system was filled with new water.

3.8. Feed formulation and preparation

3.8.1. Selection of ingredients and diet formulation

Fish meal, soybean meal, mustard oil cake, rice bran, corn meal, fish oil, wheat flour, vitamin and mineral premix were used for the preparation of feed. Vitamin and mineral premix were also used for the feed preparation. The source of pepsin was Loba Chemie. A feed containing approximately 35% protein were prepared keeping all the ingredients same except pepsin. To do this, required amount of finely ground and sieved ingredients were weighed as per formulae with a digital precision electric balance and the required amount of pepsin were added and mixed thoroughly. After mixing all the ingredients, adequate amount of water was added and converted into pellets by pelleting machine. Between preparing different doses feed, the pellet machine and related equipment were washed thoroughly to avoid any cross contamination. Then the pellets were dried under sunlight and stored in the plastic bag in air tight condition and then kept in refrigerator. After formulation of feed, proximate composition of the formulated feeds was analyzed according to standard

procedures given by Association of Official Analytical Chemists (AOAC, 1980). Proximate composition of feed showed in Table 1.

Table 1. Proximate composition of feed (% dry matter basis)

Treatment	Dry matter (%)	Protein (%)	Lipid (%)	Ash (%)	Crude fiber (%)	Moisture (%)	NFE (%)
T_1	91.07	34.24	8.19	12.08	5.44	8.93	31.12
T_2	91.01	33.89	8.33	12.10	5.09	7.99	33.60
T_3	91.06	34.56	6.04	10.26	5.34	8.34	35.46
T_4	91.11	34.17	7.68	11.90	5.33	8.89	32.52
T_5	91.18	33.58	7.53	10.88	5.27	8.72	34.72

*NFE=Nitrogen-free extract

3.8.2. Diet preparation

Feed has been formulated according to feed formulation chart by Pearson Square method (Table 2). A feed containing around 35% protein were prepared keeping all the ingredients same except pepsin enzyme. Dietary ingredients were grounded to a small particle size in a hammer mill pastel and passed through a small mesh sieve. Then all the dry ingredients individually weighted according to formula. After that dry ingredients were thoroughly mixed for 10 min. The lipid sources were made premix first. Then the lipid mixture was added to the dry ingredients and mixed for another 10 min. The required amount of water (35-40% of the dry ingredients) was added to the premixed ingredients and mixed for another 10 min. The mixture was then passed through a feed pellet machine with an appropriate diameter (1.8 mm) to prepare pellets. The pellets were dried under sunlight till the diet moisture content is reduced to about 10%. After that prepared diet was stored in the plastic bag in air tight condition and kept in refrigerator for further use.

Table 2. Formulation of experimental diets (**Pearson square**)

Ingredients	Inclusion level (%)	Protein content in ingredients (%)	Protein contents in diets CP (%)
Fish meal	35	60	21
Mustard Oil Cake	23	28	6.44
Rice Bran	9	12	1.08
Wheat Flour	9	14	1.26
Soybean meal	10	45	4.5
Corn meal	9	8	.72
Fish oil	3	-	-
Vit. Premix	1	-	-
Binder	1	-	-
Pepsin	***	-	-
Total	100.00		35.00
*** Pepsin inclusion level=(0, 0.25,0.50, 0.75, 1) g/kg feed			

3.9. Feeding rate and frequency

Fishes were fed two times in a day (at around 9.00 a.m. and 5.00 p.m. Feeding rate was 7% of total fish body weight basis. Total feed on 7% body weight basis were devided into two parts and fed the fish at morning and at afternoon. Feeds were applied directly to the experimental aquaria.

3.10. Sampling of the experimental fish

At every 15 days interval sampling was done while fish passed a 24 h starvation period before sampling to reduce waste contamination in fish body. Six fishes from each treatment (two fish samples from each replication) were collected randomly. Prior to weighing, fish were caught with a fine mesh scoop net and excess water was then removed from fish body by gently blotting on a soft tissue paper. Weighing was done by using digital precision electric balance. Then standard length and total length were measured by using scale. Blood was directly taken into the blood collection vacutainer tubes by cutting the caudal vain (in the tail portion) of fish for hematological analysis.

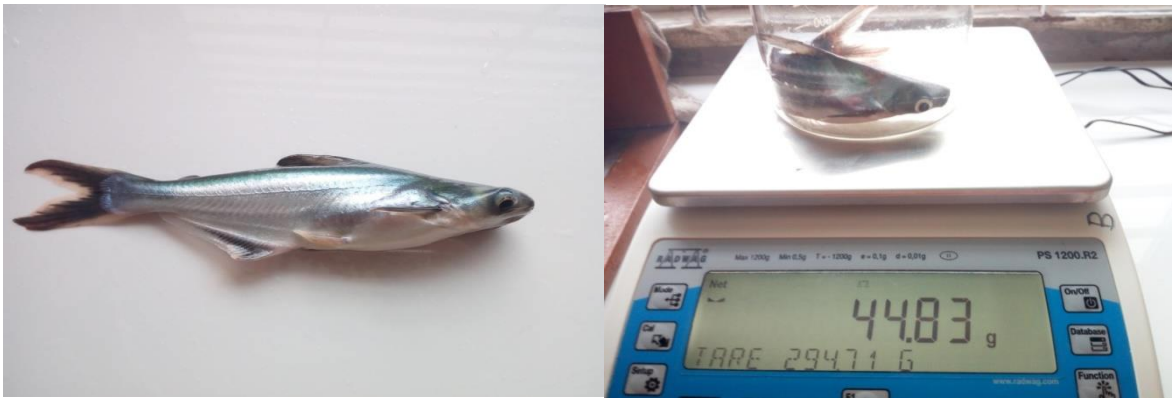

Plate-6. Weighing of fish before dissection

Plate-7. Collection of blood in vacutainer tube

Plate-8. Analysis of blood samples by hematology analyzer

3.11. Water quality parameters

The water quality parameters as water temperature, dissolved oxygen (DO), pH, ammonia, phosphate, nitrate and nitrite were monitored throughout the experimental period. Water temperature of the aquaria was observed with the help of an oxygen meter (Waterproof Tester, Model 7031). Dissolved oxygen of the water was measured by using an oxygen meter (Waterproof Tester, model: 7031). Electronic pH meter (ORP, Model 6011) was used to measure the pH of water. Ammonia, phosphate, nitrate and nitrite of the water were measured by using the photometer (Photo Flex, WTW, Germany).

3.12. Parameters studied for this experiment

3.12.1. Parameters studied for growth performance and feed utilization of fish

In order to study the effect of pepsin on the growth and feed utilization during 90 days of experiment the following parameters were determined:

a. Weight gain (g) = Mean final weight- mean initial weight.

b. Specific growth rate, SGR (% /day) = $\frac{Log_eW_2 - Log_eW_1}{T_2 - T_1} \times 100$

Where,

W1= the initial live body weight (g) at time T1 (day)

W2= the final live body weight (g) at time T2 (day)

c. Feed Conversion Efficiency (FCE) = Live weight gain (g) /Dry feed fed

d. Feed Conversion Ratio (FCR) = Dry feed fed / Live Weight gain (g)

e. Protein Efficiency Ratio (PER) = Live weight gain (g) / Crude protein intake (g)

3.12.2. Blood samples and hematological parameters

For hematological assay blood samples were collected from the caudal vein of fish. Hematological parameters such as (RBC), white blood cell (WBC), hematocrit (Hct**)**, hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin Concentration (MCHC) and mean corpuscular volume (MCV) value were determined by hematology analyzer (Hycel, Hycount 3, Austria) whereas blood glucose was measured by using glucose meter (Easy Touch GCHb Meter).

3.13. Statistical analysis

The obtained data were calculated by Microsoft Excel and statistically analyzed using IBM SPSS statistics (software version 23). Data are expressed as means ± standard error (S.E.). Any statistical significance of difference between means was tested at 95% confidence level by One-way ANOVA. Significance differences among means were determined by Duncan's multiple range test.

CHAPTER-4

RESULTS

Results are the ultimate objective of scientific research and this section consists of the observations and measurements recorded while conducting the procedures described in the methods section. Results that had found from the experiment based on growth, feed utilization and hematology parameters in *P. hypophthalmus* by providing different dietary levels of pepsin have been described providing sufficient data, figure, and information.

4.1 Water quality parameters

Different water quality parameters such as water temperature, dissolved oxygen (DO), pH, ammonia, phosphate, nitrite and nitrate were monitored and found in the desirable range according to Boyd (1979), Jhingran and Pullin (1985) and Rahman et al. (1982) throughout the experimental period. There was no indication of the adverse effect of water quality parameters on the survival and growth of *P. hypophthalmus* (Table 3).

Table 3. Physico-chemical parameters of the aquaria during the rearing period *of P. hypophthalmus*.

Sampling No.	Parameters	**T 1**	**T 2**	**T 3**	**T 4**	**T 5**
1st	Temperature (°C)	29±0.1	28.6±0.1	28.8±0.2	29±0.2	27.8±0.2
	pH	7.6	7.46	7.72	7.6	7.5
	Dissolved oxygen (mg/l)	5.9±0.1	6±0.1	5.7±0.1	6.1±0.3	6.2±0.2
	Ammonia (ppm)	0.6±0.2	0.5±0.1	0.5±0.1	0.5±0.2	0.4±0.1
	Nitrate (ppm)	2.5±0.2	2.0±0.2	1.6±0.1	1.5±0.3	1.7±0.2
	Nitrite (ppm)	0.6±0	0.5±0.1	0.4±0	0.6±0	0.4±0.1
	Phosphate (ppm)	0.2±0.1	0.2±0	0.1±0	0.2±0.1	0.2±0
2nd	Temperature (°C)	29.3±0.4	28±0.2	28.1±0.2	28±0.2	27.9±0.3
	pH	7.1	7.1	7.0	7.0	7.0
	Dissolved oxygen (mg/l)	5.7±0.2	5.5±0.2	5.6±0.2	5.5±0.2	5.3±0.1
	Ammonia (ppm)	0.5±0.2	0.5±0.2	0.6±0.1	0.7±0.1	0.5±0.1
	Nitrate (ppm)	2.0±0.2	1.5±0.2	1.8±0.2	1.4±0.2	2.2±0.1
	Nitrite (ppm)	0.6±0.1	0.5±0	0.5±0	0.5±0	0.6±0

	Phosphate (ppm)	0.2±0	0.2±0	0.1±0	0.2±0	0.2±0
3rd	Temperature (°C)	27.9±0.1	28±0.2	28±0.1	27.1±0.4	26.5±0.5
	pH	6.8	6.9	7.0	7.0	7.1
	Dissolved oxygen (mg/l)	6±0.1	5.9±0.1	6.1±0.2	5.8±0.1	5.8±0.2
	Ammonia (ppm)	0.4±0.1	0.4±0.1	0.6±0.1	0.5±0.1	0.7±0.1
	Nitrate (ppm)	1.7±0.2	1.1±0.1	1.2±0.1	1.1±0.1	1.2±0.1
	Nitrite (ppm)	0.4±0	0.4±0.1	0.7±0.1	0.7±0	0.4±0.1
	Phosphate (ppm)	0.1±0.1	0.2±0	0.1±0	0.2±0.1	0.1±0
4th	Temperature (°C)	28±0.1	27.9±0.3	28±0.2	28±0.2	27±0.4
	pH	7.0	7.1	6.9	7.2	7.3
	Dissolved oxygen (mg/l)	5.6±0.1	5.8±0.3	5.4±0.3	5.7±0.2	5.9±0.4
	Ammonia (ppm)	0.4±0.1	0.2±0	0.4±0.1	0.3±0	0.4±0.1
	Nitrate (ppm)	1.1±0.1	2.5±0.1	1.5±0.2	2.3±0.2	2.1±0.1
	Nitrite (ppm)	0.3±0.1	0.4±0	0.6±0	0.5±0	0.4±0.1
	Phosphate (ppm)	0.1±0	0.2±0.1	0.2±0	0.3±0	0.2±0.1
5th	Temperature (°C)	29.7±0.3	28.1±0.1	28±0.3	27.8±0.2	27.6±0.3
	pH	7.2	7.2	7.1	7.2	7.0
	Dissolved oxygen (mg/l)	5.7±0.2	5.5±0.3	5.6±0.2	5.7±0.3	5.6±0.1
	Ammonia (ppm)	0.7±0.2	0.2±0.1	0.5±0.2	0.4±0.1	0.5±0.2
	Nitrate (ppm)	1.7±0.1	1.2±0.1	1.6±0.1	1.2±0.1	1.4±0.3
	Nitrite (ppm)	0.7±0.1	0.4±0	0.4±0	0.5±0	0.4±0.1
6th	Temperature (°C)	27.9±0.2	27.3±0.2	25.7±0.4	26.2±0.2	24.9±0.3
	pH	7.9	8.1	8.15	7.8	7.4
	Dissolved oxygen (mg/l)	5.6±0.3	5.7±0.2	5.9± 0.3	5.6±0.3	5.9±0.1
	Ammonia (ppm)	0.6±0.1	0.5±0.2	0.3±0.1	0.5±0.2	0.4±0.1
	Nitrate (ppm)	1.1±0.1	1.8±0.1	1.7±0.1	2.3±0.2	1.0±0
	Nitrite (ppm)	0.4±0.1	0.4±0	0.5±0.1	0.5±0.1	0.4±0.1
	Phosphate (ppm)	0.2±0	0.1±0	0.2±0	0.2±0	0.1±0

4.2 Growth performance and feed utilization

The effects of dietary supplementation of pepsin enzyme on growth and feed utilization after 90 days of trial showed significant effects in fish that fed exogenous pepsin supplemented diets.

4.2.1 Growth performance

Growth parameters such as average weight gain and specific growth rate (SGR) were significantly ($p<0.05$) higher in fish fed with 0.5 g pepsin per kg feed (under treatment T_3) at 90 days experimental period compared to control group. Highest average weight gain (20.4±1.04 g) and specific growth rate (0.32± 0.02 g) was resulted from treatment-3 whereas poorest weight gain (15.43±0.59 g) and specific growth rate (0.23± 0.01 g) was found in control group. No significant ($p>0.05$) differences were found in other treatments compared to control group.

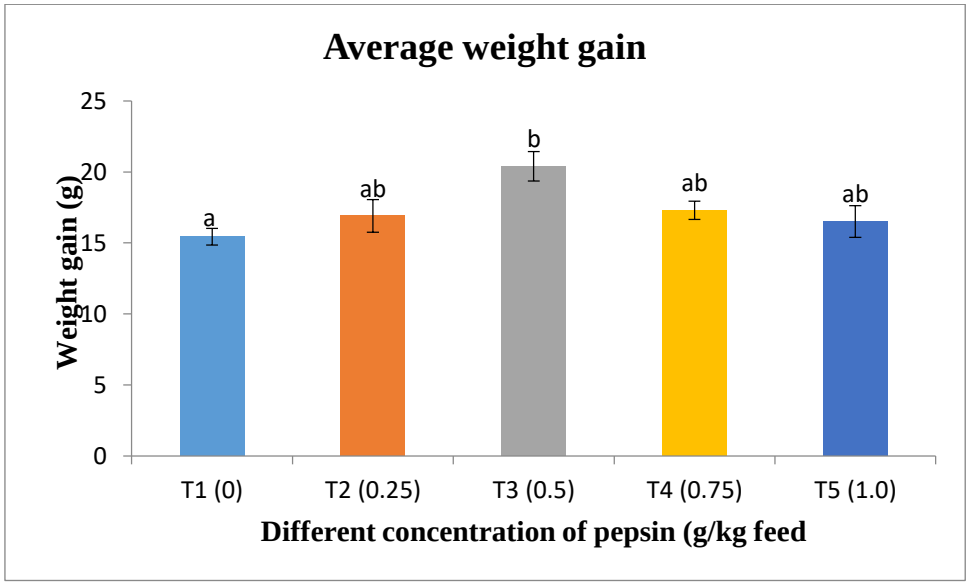

Figure 1. Average weight gain of *P. hypophthalmus* after 90 days culture period under different inclusion levels of pepsin in diet. Values with different superscripts differ significantly ($p<0.05$). (Vertical bars= ±S.E.)

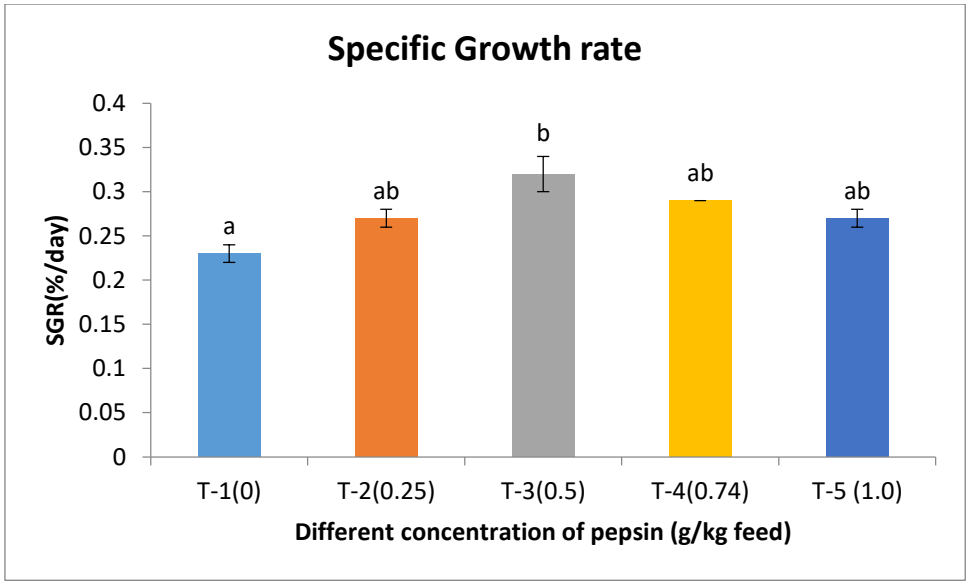

Figure 2. Specific growth rate of *P. hypophthalmus* after 90 days culture period under different inclusion levels of pepsin in diet. Values with different superscripts differ significantly ($p<0.05$). (Vertical bars= ±S.E.)

4.2.2 Feed Utilization

Among all the treatments, better feed conversion ratio (2.03±0.04) and feed conversion efficiency (0.49±0.01) were found in fish fed with 0.5 g pepsin kg^{-1} feed whereas comparatively poor feed conversion ratio (2.84±0.05) and feed conversion efficiency (0.35±0.01) were observed in control group. The experimental results showed significant ($P < 0.05$) difference in treatment-3 in case of feed conversion ratio (FCR) and feed conversion efficiency (FCE) compared to all other treatment groups. The highest protein efficiency ratio (1.36±0.04) was observed in treatment-3 and lowest result (0.95±0.04) was found in control group. There was significant difference ($p<0.05$) in protein conversion efficiency between treatment-3 and control group as well as with treatment-2.

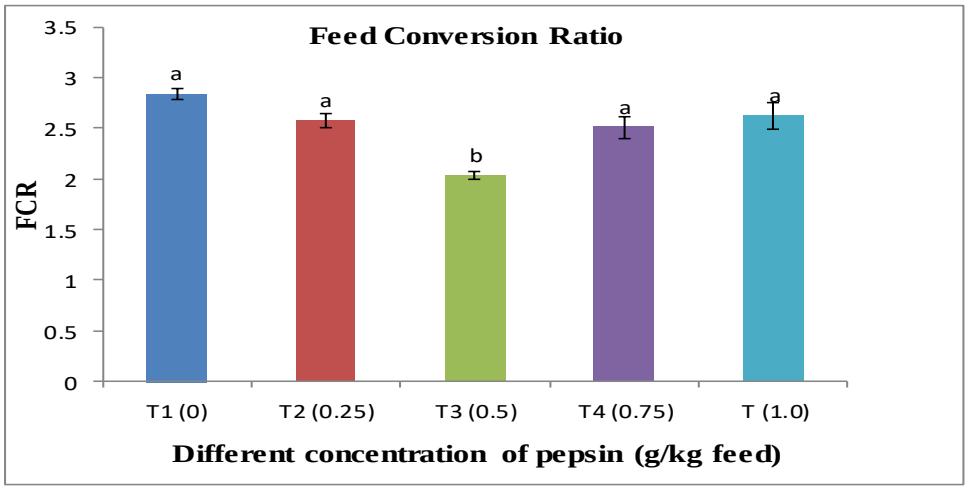

Figure 3. FCR value of *P. hypophthalmus* after 90 days culture period under different inclusion levels of pepsin in diet. Values with different superscripts differ significantly (p<0.05). (Vertical bars= ±S.E.)

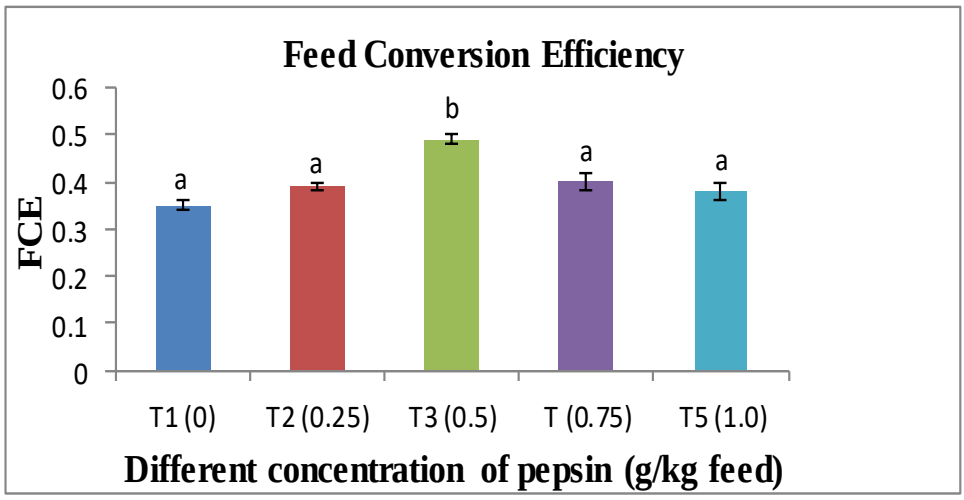

Figure 4. FCE value of *P. hypophthalmus* after 90 days culture period under different inclusion levels of pepsin in diet. Values with different superscripts differ significantly (p<0.05). (Vertical bars= ±S.E.)

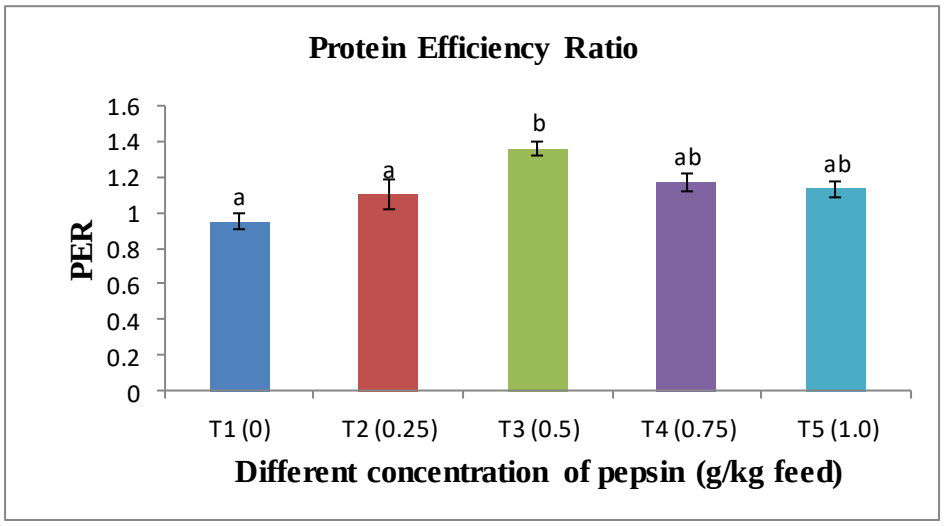

Figure 5. PER value of *P. hypophthalmus* after 90 days culture period under different inclusion levels of pepsin in diet. Values with different superscripts differ significantly (p<0.05). (Vertical bars= ±S.E.)

4.3 Hematological parameters

Hematological parameters such as red blood cells counts, white blood cells counts, hemoglobin, hematocrit, mean corpuscular volume, glucose, mean corpuscular hemoglobin, and mean corpuscular hemoglobin concentration were recorded and presented in table 4. Significantly (p<0.05) better RBC and WBC were observed in treatment-3 compared to control group. Treatment-3 showed the highest amount of RBC (2.30± 0.03×10^6 cells/µl) count and WBC (86.70 ± 0.46×10^3 cells/µl) count while control group gave the poorest RBC (1.83 ± 0.02×10^6 cells/µl) and WBC (68.60 ± 0.52×10^3 cells/µl). Statistical analysis showed significant relationship between treatment-1 and treatment-3.

The highest blood glucose level (198.5±4.54 mg/dl) was obtained in treatment-3 which was significantly (P < 0.05) higher compared to the control group (168.7±2.89 mg/dl). Fluctuations in glucose level were also observed that showed in Table 4. Results showed higher hemoglobin level (10.08±0.20 g/dl) in treatments-3 whereas control, treatment-2, treatment-4 and treatment-5 showed 8.10±0.14 g/dl, 8.35±0.31 g/dl, 8.62±0.18 g/dl, and 8.80±0.25 g/dl in turn. After 90 days feeding trial, hematocrit values were 25.2±0.67%, 26.6±0.54%, 31.2±1.14%, 28.7±0.52%, and 28.5±0.66% in treatment-1 to treatment-5 respectively. In case of hematocrit, there

were no significant differences (p>0.05) among the different treatment groups. In case of mean corpuscular volume, control showed the highest MCV value was 138.0±4.9 fl/cell after 90 days feeding trial. The MCV values observed 134.2±3.4 fl/cell, 135.7±4.1 fl/cell, 137.0±5.8 fl/cell, 137.0±1.87 fl/cell in treatment-2, treatment-3, treatment-4 and treatment-5 respectively. There were significant differences (P< 0.05) between control and treatments-2 and treatment-3. Control (T_1) showed higher mean corpuscular hemoglobin (MCH) (44.44±0.97 pg/cell) than the other treatments. Statistical analysis showed significant difference (p<0.05) between treatment-2 and the treatment-3. The highest mean corpuscular hemoglobin concentration (MCHC) (32.50±1.40 g/dL) was found in treatment-3 whereas, incases of treatment-4 the value was the lowest (30.03±0.42 g/dL). Statistical analysis showed no significant differences (P>0.05) among all the treatments.

Table 4. Mean hematological parameters of *P. hypophthalmus* at different culture period under different inclusion level of pepsin in diet. Values with different superscripts differ significantly (*P* < 0.05). (Data are presented with mean ± S.E.)

Parameters	Treatment (g/Kg feed)	Days				
		0	15	30	60	90
RBC (*10^6 cells/µl)	0	1.49 ±0.01	1.62 ±0.02	1.79 ± 0.01	2.01 ± 0.02	1.83 ± 0.02
	0.25	1.50 ±0.01	1.63 ±0.02	2.20 ± 0.02	2.58 ± 0.03	1.99 ± 0.01
	0.50	1.51 ±0.01	1.84 ±0.02	2.05 ± 0.03	2.65 ± 0.05	2.30 ± 0.03
	0.75	1.49 ±0.01	1.58 ±0.01	1.85± 0.03	2.38± 0.02	2.10± 0.02
	1.0	1.52 ±0.01	1.61 ±0.03	1.86± 0.02	2.41± 0.03	2.08± 0.02
WBC (*10^3 cells/µl)	0	61.17±0.7	63.45±0.8	65.93±0.82	67.80±0.35	68.6±0.52
	0.25	60.17±0.81	65.57±0.5	67.02±0.71	72.20±0.97	76.13±0.69
	0.50	60.90±1.60	69.40±0.7	74.45±0.96	80.33±0.69	86.70±0.46
	0.75	60.00±1.60	65.67±0.5	68.50±0.85	71.05±0.51	77.50±0.48
	1.0	60.67±0.73	64.90±0.8	66.33±0.80	75.40±0.99	77.57±0.57
Hemoglobin (g/dl)	0	7.98 ± 0.07	8.06± 0.12	7.64± 0.37	8.12± 0.03	8.10± 0.14
	0.25	8.02±0.06	7.95±0.10	8.00±0.13	7.88±0.20	8.35±0.31

	0.50	7.92±0.05	9.19±0.09	10.13±0.26	10.88±0.21	10.08±0.20
	0.75	7.90±0.04	7.59±0.11	8.05±0.23	9.19±0.19	8.62±0.18
	1.0	8.06±0.03	8.16±0.09	8.28±0.10	9.56±0.10	8.80±0.25
Hematocrit (%)	0	20.4± 0.26	22.0±0.28	24.4±0.52	33.3±0.42	25.2±0.67
	0.25	20.7±0.45	22.2±0.29	28.6±0.53	30.3±0.65	26.6±0.54
	0.50	19.9±0.36	25.0±0.50	25.5±0.56	37.9±1.32	31.2±1.14
	0.75	18.9±0.47	19.8±0.83	28.3±0.61	34.3±0.52	28.7±0.52
	1.0	20.4±0.47	22.9±0.41	27.7±0.51	32.1±0.71	28.5±0.66
Glucose (mg/dl)	0	145.2±4.41	147.0±2.53	165.8±5.23	172.8±4.92	168.7±2.89
	0.25	149.7±3.54	152.0±6.83	179.3±3.93	181.8±5.28	167.7±5.99
	0.50	148.5±3.45	154.2±4.13	227.5±5.71	240.7±5.12	198.5±4.54
	0.75	151.3±3.49	159.6±3.63	162.0±3.64	185.1±5.46	187.3±4.94
	1.0	146.8±2.61	150.3±2.43	165.5±2.49	230.0±7.52	174.2±6.74
MCV (fl/cell)	0	136.6±1.5	136.2±2.5	136.4±3.3	165.6±2.9	138.0±4.9
	0.25	138.3±3.9	136.5±1.4	129.8±2.2	117.8±3.8	134.2±3.4
	0.50	131.8±2.8	136.2±2.9	124.7±3.7	142.9±2.8	135.7±4.1
	0.75	127.0±8.0	125.3±13.9	153.4±11.1	144.1±3.1	137.1±5.8
	1.0	134.4±3.05	142.2±3.07	149.4±3.44	133.0±3.08	137.0±1.87
MCH (pg/cell)	0	53.54±0.37	49.94±0.94	42.72±2.02	40.36±0.39	44.44±0.97
	0.25	53.52±0.74	48.92±0.81	36.34±0.57	30.61±1.00	42.06±1.62
	0.50	52.42±0.27	50.04±0.56	49.50±1.70	41.19±1.43	43.84±0.81
	0.75	53.03±0.24	48.04±0.61	43.50±1.16	38.69±0.82	41.13±0.48
	1.0	53.14±0.27	50.70±1.37	44.57±0.38	39.63±0.63	42.33±0.90
MCHC (g/dL)	0	39.22±0.32	36.73±0.96	31.48±1.86	24.39±0.37	32.40±1.36
	0.25	38.79±0.81	35.84±0.54	28.01±0.33	26.05±0.90	31.48±1.54
	0.50	39.85±0.88	36.81±0.77	39.82±1.44	28.91±1.30	32.50±1.40
	0.75	41.90±1.04	38.63±1.35	28.42±0.79	26.86±0.57	30.03±0.42
	1.0	39.65±1.06	35.67±0.83	29.91±0.66	29.86±0.76	30.89±0.54

CHAPTER-5

DISCUSSION

Fish growth is the outcome of feeding, digestion, assimilation, and metabolism of food materials. Fish growth response and feed utilization were improved with enzyme supplementation, suggesting that the negative effects of plant ingredients were compensated to some extent by addition of the enzymes (Tahoun et al., 2011). The present results are in agreement with the previous studies on growth responses of fish by feeding exogenous enzyme added feed. Many researchers conducted experiments on exogenous enzymes added feed to determine the growth responses of various fish species viz. on tilapia, *Oreochromis niloticus* (Lin et al., 2007), *Pangasius pangasius* (Debnath et al., 2005), rainbow trout (Irianto et al., 2002), and common carp, *Cyprinus carpio* (Singh et al., 2011).

Present results revealed that the enzyme additive feed exhibited higher growth rate and SGR than that of free enzymes diet (control) indicated that enzyme is beneficial for the growth of fish.

The present results showed that fishes fed with 0.5 g pepsin supplemented diet (treatment-3) showed significantly ($p \leq 0.05$) higher specific growth rate and average weight gain than fish fed the control diet and other concentration of pepsin (0.25, 0.75 and 1.0 g per kg diet) enzyme. Enzyme supplementation showed significant differences between the control diet and treatment-3 in terms of FCE and PER. The results indicate that supplementing diets with exogenous protease enzymes (pepsin) significantly ($p \leq 0.05$) improves feed conversion ratio (FCR) compared to control diet.

Similar results were observed by Soares et al. (2008) stating that the inclusion of exogenous protease at 0.0, 0.05, 0.10 and 0.15% gave rise to improve FCR, WG and SGR of tucunare paca (*Cichla temensis)* juvenile and the best results were recorded at 0.1%. Moreover, Drew et al., (2005) reported a significant improvement in feed efficiency of rainbow trout (*Oncorhynchus mykiss*) by the addition of protease enzyme at 0.25% to diet of canola: Pea mixture.

Supplementation of different dietary exogenous protease enzyme (pepsin) resulted in better growth performance and feed utilization of pangus *(P. hypophthalmus).*

Treatment-3 (T3 containing 0.5g pepsin/kg feed) had the highest specific growth rate of 0.32% per day compared to control diet (0.23% per day) and others pepsin supplementary diet. The average weight gain was higher (20.4 g) in treatment-3 (T3 containing 0.5g pepsin/kg feed) compared to control diet (15.43 g) and others pepsin supplementary diet. The higher FCE (0.49) and PER (1.36) were also observed in case of treatment-3. Though the lowest feed conversion ratio also observed in treatment-3 (2.03±0.04). The reasons behind high FCR value (>2) might be the feed type (pelleted feed), feed size as well as culture system. Amin et al. (2005) recorded FCR 1.65 for pangus culture with eight earthen ponds, which is lower than the value of the present experiment.

However, other scientists achieved higher FCR that observed in the present experiment. Halder and Jahan (2001) observed FCR (2.96-3.09) in 5 months pangus polyculture with carp, which has similarity with the value of the present experiment.

According to Yildirim and Turan (2010) supplemented enzyme complex group can significantly improve growth performance and feed utilization in African catfish. Vielma et al. (2002) also recorded improved growth of rainbow trout by exogenous supplementation of phytase enzyme. Jackson et al., (1996) observed improved growth rate in channel catfish when fed with exogenous enzyme added feed. Kumar et al. (2006) demonstrated that in rohu carp, supplementation of α- amylase enzyme improved growth rate and protein utilization only when fish were fed diets with a sub-optimal protein level (27% instead of 35%). These results indicated that exogenous enzyme supplemented feed can promote fish growth.

Blood is a fluid connective tissue circulating into the fish body. It provides one of the methods of communication between the cells of different parts of the body. The study of the fish blood parameters are important for determining factors related to its physiological capacity (Affonso, 2001; Wells et. al., 2005).

In this study, RBC, WBC, hemoglobin, hematocrit, and glucose were significantly higher in enzyme supplemented group, treatment -3 with 0.50 g pepsin per kg feed and other treatments containing enzyme shown better results than control group.

Hematological parameters in fish are associated to physiological and immune status (Yarahmadi et al., 2014). The results of the present study indicated that

administration of feeding with pepsin supplemented diet increases the WBC, RBC, hemoglobin etc. In agreement with our results, Ebrahimi et al. (2012) reported that common carp fingerlings fed dietary immunogen had higher level of WBC. The white blood cells are one of the innate immune defenses of fish and the increase of WBC levels indicates the immunostimulatory effect of pepsin enzyme.

The analysis of hematological parameters showed significant effect ($p<0.05$) of protease enzyme (pepsin) supplementation on blood parameters. The values of WBC and differential cell counts in the present study are also similar to the values obtained by Oscar et al. (2016) for normal healthy *P. hypophthalmus* cultured in recirculatory aquaculture system (RBC 1.79-2.75x10^6/µL, WBC 36.3-94.3 x10^3/µL, MCV 106.3-156.6 fL, glucose 87-138 mg/dL).

Thus, it is clear from the present study that protease increased the availability of proteins by its proteolytic activity leading to improve growth performance and feed utilization of pangus (*P. hypophthalmus*). Therefore, it can be concluded that exogenous protease enzyme (pepsin) supplementation in diets can enhance the growth and feed utilization as well as keeps the fish in good physiological condition.

CHAPTER-6

CONCLUSIONS

Fish is an ideal source of protein and cheaper than any other food protein. Through the whole world it plays an important role in nutrition. The people of Bangladesh depend on fish as the principal source of animal protein. Aquaculture is recognized as the fastest growing agri-business sector and has thus become an important component of global food supply. It has great economic importance because of its good flesh. Flesh of mature and older fish contains thick layer of fat. The liver of this fish contains vitamin "A". However in the recent years, economic benefit from this farming is being depleted partly due to increasing feed cost, lack of proper management, unavailability of low cost supplementary feeds and some socio-economic constraints. Upon success of this experiment, people can understant about the importance of using pepsin in better feed utilization and rapid growth of *Pangasius hypophthalmus* to produce good quality fish. It could be concluded that the exogenous dietary supplementation of pepsin can be used safely and economically to improve protein digestibility and reduce protein content of *P. hypophthalmus* diet with maintaining the growth performance and good physiological state.

CHAPTER-7

RECOMMENDATIONS AND FUTURE PERSPECTIVE

The aim of this study was to know the the importance of using pepsin in better feed utilization and rapid growth of *Pangasius hypophthalmus* which will be helpful for the pangus fish farming. Although a qualitative approach was followed to explore the objectives of the research, there are some limitations of the study which can be minimized by following the recommendation:

- This research can be conducted in cisterns rather than aquaria to get better results.
- Further trail can be conducted to confirm the results and experimental error.
- Lime and salt can be used, either for prevention or early control of disease before it becomes too serious.
- For better hematological parameters the blood sample should be collected and preserved immediately after collection and blood parameters should be taken as early as possible.

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CHAPTER-8

REFERENCES

Affonso EG. 2001. Respiratory characteristics of *Hoplosternum littorale* (Siluriformes, Callichthyidae). Acta Amazonica 31: 249–262.

Adeoye AA, Jaramillo-Torres A, Fox SW, Merrifield DL, Davies SJ. 2016. Supplementation of formulated diets for tilapia (*Oreochromis niloticus*) with selected exogenous enzymes: Overall performance and effects on intestinal histology and microbiota. Animal Feed Science Technology, 215: 133-143.

Amin AKMR, Bapary MAJ, Islam MS,Shahjahan M, Hossain MAR. 2005. "The Impacts of Compensatory Growth on Food Intake, Growth Rate and Efficiency of Feed Utilization in Thai Pangas (*Pangasius hypophthalmus*)", Pakistan Journal of Biological Science. 8(5): 766-770.

ADB. 2005. An evaluation of small-scale freshwater rural aquaculture development for poverty reduction. Operation Evaluation Department, Asian Development Bank, Manila, Philippines. 35 pp.

Acamovic T. 2001. Commercial application of enzyme technology for poultry production. World's Poultry Science Journal. 57(3): 225–242.

BFTI (Bangladesh Foreign Trade Institute). 2016. A Study on sector-based need assessment of Business Promotion Council: Fisheries Products. Dhaka

Bedford MR, Schulze H 1998. Exogenous enzymes in pigs and poultry. Nutrition Research Review. 11: 91–114.

Boyd CE. 1979. Water Quality in Warm water Fish Ponds, Agriculture Experiment Station, Auburn, Alabama, pp 359.

Castillo S, Gatlin DM. 2015. Dietary supplementation of exogenous carbohydrase enzymes in fish nutrition: A Review of Aquaculture Research. 25: 37-46.

Debnath D, Sahu NP, Pal AK, Jain KK, Yengkokpam S, Mukherjee SC. 2005. Mineral status of *Pangasius pangasius* (Hamilton) fingerlings in relation to supplemental phytase: Absorption, whole body and bone mineral content, Aquaculture Research. 36: 326-335.

Dabrowska H, Grudniewski C, Dabrowski K. 1979. Artificial diets for common carp, effect of the addition of enzyme extracts. The Progressive Fish Culturist. 41: 196–200.

Debnath D, Pal AK, Sahu NP, Jain KK, Yengkokpam S, Mukherjee SC. 2005. Effect of dietary microbial phytase supplementation on growth and nutrient digestibility of *Pangasius pangasius* (Hamilton) fingerlings. Aquaculture Research. 36: 180–187.

Davies SJ, Gouveia A. 2008. Enhancing the nutritional value of pea seed meals (*Pisum sativum*) by thermal treatment or specific isogenic selection with comparison to soybean meal for African catfish, *Clarias gariepinus*. Aquaculture 283(1– 4): 116 – 122.

Drew MD, Racz VJ, Gauthier R, Thiessen DL. 2005. Effect of adding protease to coextruded flax: Pea or canola: Pea products on nutrient digestibility and growth performance of rainbow trout (*Oncorhynchus mykiss*). Animal Feed Science Technology. 119: 117-128.

Dey MM, Alam MF, Bose ML. 2010. Demand for aquaculture development perspectives from Bangladesh for improved planning. Reviews in Aquaculture 2: 16–32.

DoF. 2016. National fish week, compendium (In Bengali). Dhaka: Department of Fisheries, Ministry of Fisheries and Livestock, Government of Bangladesh.

Ebrahimi G, Oraji H, Khalesi M, Sudagar M, Barari A, Dangesaraki MZ, Khalili KJ. 2012. Effects of a prebiotic, Immunogen®, on feed utilization, body composition, immunity and resistance to Aeromonas hydrophila infection in the common carp *Cyprinus carpio* (Linnaeus) fingerlings. Journal of Animal Physiology and Animal Nutrition 96: 591-599.

FRSS. 2017. Yearbook of Fisheries Statistics of Bangladesh. Fisheries Resources Survey System (FRSS), Department of Fisheries, Bangladesh. 33: 124.

FRSS. 2012. Fisheries Statistical Yearbook of Bangladesh. Fisheries Resources Survey System, Department of Fisheries, Bangladesh. 28: 46.

Felix N, Selvaraj S. 2004. Enzyme for sustainable aquaculture. Aquaculture Asia 1: 5–6.

FAO. 2016. The State of World Fisheries and Aquaculture 2016. Contributing to food security and nutrition for all. Rome. 200 pp.

Farhangi M, Carter CG. 2007. Effect of enzyme supplementation to dehulled lupin-based diets on growth, feed efficiency, nutrient digestibility and carcass composition of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Research. 38: 1274–1282.

Giri SS, Sahoo SK, Sahu AK, Meher PK. 2003. Effect of dietary protein level on growth, survival, feed utilisation and body composition of hybrid Clarias catfish (*Clarias batrachus* × *Clarias gariepinus*). Animal Feed Science Technology. 104: 169–178.

Goda AM, El-Haroun ER, Kabir Chowdhury MA. 2007. Effect of totally or partially replacing of fish meal by alternative protein sources on growth of African catfish *Clarias gariepinus* (Burchell, 1822) reared in concrete tanks. Aquaculture Research. 38(3): 279–287.

Ghose B. 2014. Fisheries and aquaculture in Bangladesh: Challenges and opportunities. Annals of Aquaculture and Research. 1: 1001.

Hekimoglu MA, Guner Y, Yavuz S, Akcan G, Gulec F. 2014. Farming of Pangus for Sustainable Aquaculture. Journal of Science and Technology. 2(1): 47-54.

Hussain MG, Mazid MA. 2001. Genetic improvement and conservation of carp species in Bangladesh. Bangladesh Fisheries Research Institute and International Center for Living Aquatic Resources Management.

Haldar GC, Jahan DA. 2001. "Polyculture of Pangus with Carp at Low Density in a Farmer's Pond", Final Report On Post Flood Rehabilitation and Adaptive Research Support Project" Bangladesh Fisheries Research Institute, Chandpur and Bangladesh Agricultural Research Council, Dhaka, 66-74 pp.

Irianto A, Austin B. 2002. Use of probiotics to control furunculosis in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Journal of Fish Diseases. 25: 333-342.

Jackson LS, Li MH, Robinson EH. 1996. Use of microbial phytase in channel catfish *Ictalurus punctatus* diets to improve utilization of phytase phosphorus. Journal of the World Aquaculture Society. 27: 309-313.

Jhingran VG, Pullin RSV. 1985. A hatchery manual for the common Chinese and Indian major carps. ICLARM studies and reviewers. 11: 191.

Jahan KM, Ahmed M, Belton B. 2010. The impacts of aquaculture development on food security: lessons from Bangladesh. Aquaculture Research. 41: 481–495.

Khalil M, Azmat H, Khan N, Javid A, Hussain A, Hussain SM, Ullah A, Abbas S. 2018. Growth Responses of Striped Catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) to Exogenous Enzyme Added Feed. Pakistan Journal of Zoology. 50(2): 685-693.

Kolkovski S, Tandler A, kissil WM, Gertler A. 1993. The effects of exogenous digestive enzymes on ingestion, assimilation, growth and survival of gill head bream (*Sparus aurata*, Sparidae, Linnaeus) larvae. Fish Physiology and Biochemistry. 12: 203-209.

Kumar S, Sahu N, Pal A, Choudhury D, Mukherjee S. 2006. Non-gelatinized corn supplemented with α-amylase at sub-optimum protein level enhances the growth of *Labeo rohita* (Hamilton) fingerlings. Aquaculture Research. 37: 284–292.

Kumar V, Sinha Ak, Makkar HP, De Boeck G, Becker K. 2012. Phytate and phytase in fish nutrition. Journal of Animal Physiology and Animal Nutrition. 96: 335-364.

Lanair D, D'Agaro E, Turri C. 1998.Use of nonlinear regression to evaluate the effects of phytase enzyme treatment of plant protein diets for rainbow trout (*Oncorhynchus mykiss*). Aquaculture. 161: 345-356.

Lin S, Mai K, Tan B. 2007. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in tilapia, *Oreochromis niloticus* × *O. aureus*, Aquaculture Research. 38: 1645-1653.

Naela M, Ragaa, Nermeen M, Abu Elala, Azza M, Kamel NF. 2017. Effect of a Serine-protease on Performance Parameters and Protein Digestibility of Cultured *Oreochromis niloticus* Fed Diets with Different Protein Levels. Pakistan Journal of Nutrition. 16: 148-154.

Odetallah NH, Wang JJ, Garlich JD, Shih JCH. 2005. Versazyme supplementation of broiler diets improves market growth performance. Poultry Science. 84: 858– 864.

Oscar AG, David DK, Stephen AS, Daniel PT, Joseph DE, Robert CW. 2016. Baseline hematology and blood chemistry of striped catfish, *Pangasius hypophthalmus*. VA: International Association for Aquatic Animal Medicine.

Refstie S, Svihus B, Shearer KD, Storebakken T. 1999. Nutrient digestibility in Atlantic salmon and broiler chickens related to viscosity and non-starch polysaccharide content in different soyabean products. Animal Feed Science Technology. 79: 331– 345.

Rahman MS, Chowdhury MY, Haque AKMA, Haq MS. 1982. Limnological studies of four fishponds. Bangladesh Journal of Fisheries Research. 2-5 (1-2): 25-35.

Sunde J, Eiane SA, Rustad A, Jensen HB, Opstvedt J, Nygard E, Venturini G, Rungruangsak-torrissen K. 2004. Effect of fish feed processing conditions on digestive protease activities, free amino acid pools, feed conversion efficiency and growth in Atlantic salmon (*Salmo salar* L.). Aquaculure Nutrition. 10: 261-277.

Soares EC, Filho MP, Roubach R, Soares e Silva RC. 2008. Exogenous protease in diets for tucunare paca (*Cichla* sp.) juvenile. Revista Brasileira Zootecnia, 37: 971-976, (In Portuguese).

Soltan MA. 2009. Effect of dietary fish meal replacement by poultry byproduct meal with different grain source and enzyme supplementation on performance, feces recovery, body composition and nutrient balance of Nile tilapia. Pakistan Journal of Nutrition 8(4): 395–407.

Singh P, Maqsood S, Samoon MH, Phulia V, Danish M, Chalal RS. 2011. Exogenous supplementation of papain as growth promoter in diet of fingerlings of *Cyprinus carpio*, International Aquatic Research 3: 1-9.

Tahounl AM, Mabroke RS, El-Haroun, ER, Suloma A. 2011. Effect of exogenous enzyme supplementation on reproductive performance of broodstock Nile

tilapia reared in a hapa-in-pond hatchery system. Egyptian Journal of Aquatic Biology and Fisheries. 15: 61-73.

Vielma J, Ruohonen K, Peisker M. 2002. Dephytinization of two soy proteins increases phosphorus and protein utilization by rainbow trout, *Oncorhynchus mykiss*. Aquaculture. 204: 145-156.

Wells RMG, Baldwins J, Seymour RS, Christian K, Brittain T. 2005. Red blood cell function and haematology in two tropical freshwater fishes from Australia. Comparative Biochemistry and Physiology. 141: 87-93.

Yarahmadi P, Miandare HK, Hoseinifar SH, Gheysvandi N, Akbarzadeh A. 2014. The effects of stocking density on hemato-immunological and serum biochemical parameters of rainbow trout (*Oncorhynchus mykiss*). Aquaculture International 23: 55-63.

Yildirim YB, Turan F. 2010. Effects of exogenous enzyme supplementation in diets on growth and feed utilization in African cat fish, *Clarias gariepinus*. Journal of Animal and Veterinary Advances. 9: 327-331.

Zamini AA, kanani HG, Esmaeili AA, Ramezani S, Zoriezahra SJ. 2014. Effects of two dietary exogenous multi-enzyme supplementation, Natuzyme® and beta-mannanase (Hemicell®), on growth and blood parameters of Caspian salmon (*Salmo trurra caspius*). Comparative Clinical Pathology. 23: 187-192.

APPENDICES

Appendix I. Growth performance and feed utilization of *P. hypophthalmus* under different pepsin doses.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
SGR	Between Groups	.010	4	.003	5.522	.013
	Within Groups	.005	10	.000		
	Total	.015	14			
FCR	Between Groups	1.061	4	.265	12.184	.001
	Within Groups	.218	10	.022		
	Total	1.278	14			
Weight gain	Between Groups	42.231	4	10.558	3.991	.035
	Within Groups	26.453	10	2.645		
	Total	68.684	14			
FCE	Between Groups	.034	4	.008	15.017	.000
	Within Groups	.006	10	.001		
	Total	.039	14			
PER	Between Groups	.267	4	.067	7.900	.004
	Within Groups	.084	10	.008		
	Total	.351	14			

Post Hoc Tests

Multiple Comparisons

Dependent Veriable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
SGR Tukey HSD	1	2	-.03333	.01751	.374	-.0910	.0243
		3	-.08000*	.01751	.007	-.1376	-.0224
		4	-.05000	.01751	.098	-.1076	.0076
		5	-.03333	.01751	.374	-.0910	.0243
	2	1	.03333	.01751	.374	-.0243	.0910
		3	-.04667	.01751	.130	-.1043	.0110
		4	-.01667	.01751	.870	-.0743	.0410
		5	.00000	.01751	1.000	-.0576	.0576
	3	1	.08000*	.01751	.007	.0224	.1376

		2	.04667	.01751	.130	-.0110	.1043
		4	.03000	.01751	.468	-.0276	.0876
		5	.04667	.01751	.130	-.0110	.1043
	4	1	.05000	.01751	.098	-.0076	.1076
		2	.01667	.01751	.870	-.0410	.0743
		3	-.03000	.01751	.468	-.0876	.0276
		5	.01667	.01751	.870	-.0410	.0743
	5	1	.03333	.01751	.374	-.0243	.0910
		2	.00000	.01751	1.000	-.0576	.0576
		3	-.04667	.01751	.130	-.1043	.0110
		4	-.01667	.01751	.870	-.0743	.0410
FCR Tukey HSD	1	2	.26667	.12044	.250	-.1297	.6631
		3	.80667*	.12044	.000	.4103	1.2031
		4	.33000	.12044	.117	-.0664	.7264
		5	.21333	.12044	.438	-.1831	.6097
	2	1	-.26667	.12044	.250	-.6631	.1297
		3	.54000*	.12044	.008	.1436	.9364
		4	.06333	.12044	.983	-.3331	.4597
		5	-.05333	.12044	.991	-.4497	.3431
	3	1	-.80667*	.12044	.000	-1.2031	-.4103
		2	-.54000*	.12044	.008	-.9364	-.1436
		4	-.47667*	.12044	.018	-.8731	-.0803
		5	-.59333*	.12044	.004	-.9897	-.1969
	4	1	-.33000	.12044	.117	-.7264	.0664
		2	-.06333	.12044	.983	-.4597	.3331
		3	.47667*	.12044	.018	.0803	.8731
		5	-.11667	.12044	.863	-.5131	.2797
	5	1	-.21333	.12044	.438	-.6097	.1831
		2	.05333	.12044	.991	-.3431	.4497
		3	.59333*	.12044	.004	.1969	.9897
		4	.11667	.12044	.863	-.2797	.5131
Weight gain Tukey HSD	1	2	-1.4333	1.3280	.813	-5.804	2.937
		3	-5.0000*	1.3280	.024	-9.371	-.629
		4	-1.9000	1.3280	.624	-6.271	2.471
		5	-1.1000	1.3280	.916	-5.471	3.271
	2	1	1.4333	1.3280	.813	-2.937	5.804
		3	-3.5667	1.3280	.126	-7.937	.804
		4	-.4667	1.3280	.996	-4.837	3.904
		5	.3333	1.3280	.999	-4.037	4.704

	3	1	5.0000*	1.3280	.024	.629	9.371
		2	3.5667	1.3280	.126	-.804	7.937
		4	3.1000	1.3280	.211	-1.271	7.471
		5	3.9000	1.3280	.086	-.471	8.271
	4	1	1.9000	1.3280	.624	-2.471	6.271
		2	.4667	1.3280	.996	-3.904	4.837
		3	-3.1000	1.3280	.211	-7.471	1.271
		5	.8000	1.3280	.972	-3.571	5.171
	5	1	1.1000	1.3280	.916	-3.271	5.471
		2	-.3333	1.3280	.999	-4.704	4.037
		3	-3.9000	1.3280	.086	-8.271	.471
		4	-.8000	1.3280	.972	-5.171	3.571
FCE Tukey HSD	1	2	-.036667	.019333	.378	-.10029	.02696
		3	-.140000*	.019333	.000	-.20363	-.07637
		4	-.047000	.019333	.184	-.11063	.01663
		5	-.029000	.019333	.585	-.09263	.03463
	2	1	.036667	.019333	.378	-.02696	.10029
		3	-.103333*	.019333	.002	-.16696	-.03971
		4	-.010333	.019333	.981	-.07396	.05329
		5	.007667	.019333	.994	-.05596	.07129
	3	1	.140000*	.019333	.000	.07637	.20363
		2	.103333*	.019333	.002	.03971	.16696
		4	.093000*	.019333	.005	.02937	.15663
		5	.111000*	.019333	.001	.04737	.17463
	4	1	.047000	.019333	.184	-.01663	.11063
		2	.010333	.019333	.981	-.05329	.07396
		3	-.093000*	.019333	.005	-.15663	-.02937
		5	.018000	.019333	.879	-.04563	.08163
	5	1	.029000	.019333	.585	-.03463	.09263
		2	-.007667	.019333	.994	-.07129	.05596
		3	-.111000*	.019333	.001	-.17463	-.04737
		4	-.018000	.019333	.879	-.08163	.04563
PER Tukey HSD	1	2	-.154000	.075004	.310	-.40084	.09284
		3	-.414667*	.075004	.002	-.66151	-.16782
		4	-.224667	.075004	.079	-.47151	.02218
		5	-.188000	.075004	.165	-.43484	.05884
	2	1	.154000	.075004	.310	-.09284	.40084
		3	-.260667*	.075004	.038	-.50751	-.01382

		4	-.070667	.075004	.874	-.31751	.17618
		5	-.034000	.075004	.990	-.28084	.21284
	3	1	.414667*	.075004	.002	.16782	.66151
		2	.260667*	.075004	.038	.01382	.50751
		4	.190000	.075004	.159	-.05684	.43684
		5	.226667	.075004	.076	-.02018	.47351
	4	1	.224667	.075004	.079	-.02218	.47151
		2	.070667	.075004	.874	-.17618	.31751
		3	-.190000	.075004	.159	-.43684	.05684
		5	.036667	.075004	.987	-.21018	.28351
	5	1	.188000	.075004	.165	-.05884	.43484
		2	.034000	.075004	.990	-.21284	.28084
		3	-.226667	.075004	.076	-.47351	.02018
		4	-.036667	.075004	.987	-.28351	.21018

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

SGR

			Subset for alpha = 0.05	
	Treatment	N	1	2
Tukey HSD[a]	1	3	.2367	
	2	3	.2700	.2700
	5	3	.2700	.2700
	4	3	.2867	.2867
	3	3		.3167
	Sig.		.098	.130
Tukey B[a]	1	3	.2367	
	2	3	.2700	.2700
	5	3	.2700	.2700
	4	3	.2867	.2867
	3	3		.3167

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

FCE

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	3	.35333	
	5	3	.38233	
	2	3	.39000	
	4	3	.40033	
	3	3		.49333
	Sig.		.184	1.000
Tukey B[a]	1	3	.35333	
	5	3	.38233	
	2	3	.39000	
	4	3	.40033	
	3	3		.49333

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

PER

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	3	.94533	
	2	3	1.09933	
	5	3	1.13333	1.13333
	4	3	1.17000	1.17000
	3	3		1.36000
	Sig.		.079	.076
Tukey B[a]	1	3	.94533	
	2	3	1.09933	
	5	3	1.13333	
	4	3	1.17000	1.17000
	3	3		1.36000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Weight gain

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	3	15.433	
	5	3	16.533	16.533
	2	3	16.867	16.867
	4	3	17.333	17.333
	3	3		20.433
	Sig.		.624	.086
Tukey B[a]	1	3	15.433	
	5	3	16.533	16.533
	2	3	16.867	16.867
	4	3	17.333	17.333
	3	3		20.433

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 3.000.

Appendix II. Hematological parameters of *P. hypophthalmus* under different pepsin doses.

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WBC	Between Groups	1275.083	4	318.771	7.472	.000
	Within Groups	6186.355	145	42.665		
	Total	7461.438	149			
RBC	Between Groups	1.739	4	.435	3.743	.006
	Within Groups	16.848	145	.116		
	Total	18.587	149			
HGB	Between Groups	55.155	4	13.789	26.926	.000
	Within Groups	74.256	145	.512		
	Total	129.411	149			
HCT	Between Groups	137.538	4	34.384	1.253	.291
	Within Groups	3977.977	145	27.434		
	Total	4115.515	149			
MCV	Between Groups	2264.938	4	566.234	4.277	.003
	Within Groups	19198.210	145	132.401		
	Total	21463.148	149			
MCH	Between Groups	450.878	4	112.719	2.950	.022
	Within Groups	5540.259	145	38.209		
	Total	5991.136	149			
MCHC	Between Groups	210.682	4	52.670	1.789	.134
	Within Groups	4269.651	145	29.446		
	Total	4480.333	149			
Glucose	Between Groups	20148.840	4	5037.210	7.155	.000
	Within Groups	102078.500	145	703.990		
	Total	122227.340	149			

Post Hoc Tests

Multiple Comparisons

Dependent Variable	(I) Treatment	(J) Treatment	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
WBC Tukey HSD	1	2	-2.8267	1.6865	.452	-7.485	1.832
		3	-8.9667*	1.6865	.000	-13.625	-4.308
		4	-3.1533	1.6865	.338	-7.812	1.505
		5	-3.5833	1.6865	.215	-8.242	1.075
	2	1	2.8267	1.6865	.452	-1.832	7.485
		3	-6.1400*	1.6865	.003	-10.799	-1.481
		4	-.3267	1.6865	1.000	-4.985	4.332
		5	-.7567	1.6865	.992	-5.415	3.902
	3	1	8.9667*	1.6865	.000	4.308	13.625
		2	6.1400*	1.6865	.003	1.481	10.799
		4	5.8133*	1.6865	.007	1.155	10.472
		5	5.3833*	1.6865	.015	.725	10.042
	4	1	3.1533	1.6865	.338	-1.505	7.812
		2	.3267	1.6865	1.000	-4.332	4.985
		3	-5.8133*	1.6865	.007	-10.472	-1.155
		5	-.4300	1.6865	.999	-5.089	4.229
	5	1	3.5833	1.6865	.215	-1.075	8.242
		2	.7567	1.6865	.992	-3.902	5.415
		3	-5.3833*	1.6865	.015	-10.042	-.725
		4	.4300	1.6865	.999	-4.229	5.089
RBC Tukey HSD	1	2	-.23233	.08801	.069	-.4755	.0108
		3	-.32300*	.08801	.003	-.5661	-.0799
		4	-.13233	.08801	.562	-.3755	.1108
		5	-.15000	.08801	.435	-.3931	.0931
	2	1	.23233	.08801	.069	-.0108	.4755
		3	-.09067	.08801	.841	-.3338	.1525
		4	.10000	.08801	.787	-.1431	.3431
		5	.08233	.08801	.883	-.1608	.3255
	3	1	.32300*	.08801	.003	.0799	.5661
		2	.09067	.08801	.841	-.1525	.3338
		4	.19067	.08801	.198	-.0525	.4338
		5	.17300	.08801	.288	-.0701	.4161

	4	1	.13233	.08801	.562	-.1108	.3755
		2	-.10000	.08801	.787	-.3431	.1431
		3	-.19067	.08801	.198	-.4338	.0525
		5	-.01767	.08801	1.000	-.2608	.2255
	5	1	.15000	.08801	.435	-.0931	.3931
		2	-.08233	.08801	.883	-.3255	.1608
		3	-.17300	.08801	.288	-.4161	.0701
		4	.01767	.08801	1.000	-.2255	.2608
HGB Tukey HSD	1	2	-.06033	.18477	.998	-.5707	.4501
		3	-1.65967*	.18477	.000	-2.1701	-1.1493
		4	-.29033	.18477	.518	-.8007	.2201
		5	-.59233*	.18477	.014	-1.1027	-.0819
	2	1	.06033	.18477	.998	-.4501	.5707
		3	-1.59933*	.18477	.000	-2.1097	-1.0889
		4	-.23000	.18477	.725	-.7404	.2804
		5	-.53200*	.18477	.037	-1.0424	-.0216
	3	1	1.65967*	.18477	.000	1.1493	2.1701
		2	1.59933*	.18477	.000	1.0889	2.1097
		4	1.36933*	.18477	.000	.8589	1.8797
		5	1.06733*	.18477	.000	.5569	1.5777
	4	1	.29033	.18477	.518	-.2201	.8007
		2	.23000	.18477	.725	-.2804	.7404
		3	-1.36933*	.18477	.000	-1.8797	-.8589
		5	-.30200	.18477	.478	-.8124	.2084
	5	1	.59233*	.18477	.014	.0819	1.1027
		2	.53200*	.18477	.037	.0216	1.0424
		3	-1.06733*	.18477	.000	-1.5777	-.5569
		4	.30200	.18477	.478	-.2084	.8124
HCT Tukey HSD	1	2	-.65967	1.35239	.988	-4.3955	3.0762
		3	-2.87300	1.35239	.215	-6.6088	.8628
		4	-.96633	1.35239	.953	-4.7022	2.7695
		5	-1.28967	1.35239	.875	-5.0255	2.4462
	2	1	.65967	1.35239	.988	-3.0762	4.3955
		3	-2.21333	1.35239	.477	-5.9492	1.5225
		4	-.30667	1.35239	.999	-4.0425	3.4292
		5	-.63000	1.35239	.990	-4.3658	3.1058
	3	1	2.87300	1.35239	.215	-.8628	6.6088
		2	2.21333	1.35239	.477	-1.5225	5.9492
		4	1.90667	1.35239	.622	-1.8292	5.6425

		5	1.58333	1.35239	.768	-2.1525	5.3192
	4	1	.96633	1.35239	.953	-2.7695	4.7022
		2	.30667	1.35239	.999	-3.4292	4.0425
		3	-1.90667	1.35239	.622	-5.6425	1.8292
		5	-.32333	1.35239	.999	-4.0592	3.4125
	5	1	1.28967	1.35239	.875	-2.4462	5.0255
		2	.63000	1.35239	.990	-3.1058	4.3658
		3	-1.58333	1.35239	.768	-5.3192	2.1525
		4	.32333	1.35239	.999	-3.4125	4.0592
MCV Tukey HSD	1	2	11.2209*	2.9710	.002	3.014	19.428
		3	8.2949*	2.9710	.046	.088	16.502
		4	5.1675	2.9710	.413	-3.040	13.375
		5	3.3429	2.9710	.793	-4.864	11.550
	2	1	-11.2209*	2.9710	.002	-19.428	-3.014
		3	-2.9259	2.9710	.862	-11.133	5.281
		4	-6.0534	2.9710	.254	-14.260	2.154
		5	-7.8779	2.9710	.067	-16.085	.329
	3	1	-8.2949*	2.9710	.046	-16.502	-.088
		2	2.9259	2.9710	.862	-5.281	11.133
		4	-3.1275	2.9710	.830	-11.335	5.080
		5	-4.9520	2.9710	.458	-13.159	3.255
	4	1	-5.1675	2.9710	.413	-13.375	3.040
		2	6.0534	2.9710	.254	-2.154	14.260
		3	3.1275	2.9710	.830	-5.080	11.335
		5	-1.8245	2.9710	.973	-10.032	6.383
	5	1	-3.3429	2.9710	.793	-11.550	4.864
		2	7.8779	2.9710	.067	-.329	16.085
		3	4.9520	2.9710	.458	-3.255	13.159
		4	1.8245	2.9710	.973	-6.383	10.032
MCH Tukey HSD	1	2	3.9104	1.5960	.108	-.4984	8.319
		3	-1.1988	1.5960	.944	-5.6077	3.209
		4	1.3194	1.5960	.922	-3.0893	5.728
		5	.1260	1.5960	1.000	-4.2827	4.534
	2	1	-3.9104	1.5960	.108	-8.3192	.498
		3	-5.1092*	1.5960	.014	-9.5181	-.700
		4	-2.5909	1.5960	.485	-6.9997	1.817
		5	-3.7843	1.5960	.129	-8.1931	.624
	3	1	1.1988	1.5960	.944	-3.2099	5.607
		2	5.1092*	1.5960	.014	.7004	9.518

		4	2.5183	1.5960	.514	-1.8904	6.927
		5	1.3249	1.5960	.921	-3.0838	5.733
	4	1	-1.3194	1.5960	.922	-5.7283	3.089
		2	2.5909	1.5960	.485	-1.8179	6.999
		3	-2.5183	1.5960	.514	-6.9271	1.890
		5	-1.1934	1.5960	.945	-5.6022	3.215
	5	1	-.1260	1.5960	1.000	-4.5349	4.282
		2	3.7843	1.5960	.129	-6.2449	8.193
		3	1.3249	1.5960	.921	-5.7337	3.083
		4	1.1934	1.5960	.945	-3.2154	5.602
MCHC Tukey HSD	1	2	.8116	1.4010	.978	-3.0586	4.682
		3	-2.7364	1.4010	.294	-6.6068	1.133
		4	-.3232	1.4010	.999	-4.1936	3.547
		5	-.3538	1.4010	.999	-4.224	3.516
	2	1	-.8116	1.4010	.978	-4.6820	3.058
		3	-3.5481	1.4010	.089	-7.4185	.322
		4	-1.1349	1.4010	.927	-5.0053	2.735
		5	-1.1655	1.4010	.920	-5.0359	2.704
	3	1	2.7364	1.4010	.294	-1.1339	6.606
		2	3.5481	1.4010	.089	-.3222	7.418
		4	2.4131	1.4010	.424	-1.4572	6.283
		5	2.3825	1.4010	.437	-1.4877	6.252
	4	1	.3232	1.4010	.999	-3.5471	4.193
		2	1.1349	1.4010	.927	-3.5471	5.005
		3	-2.4131	1.4010	.424	-2.7354	1.457
		5	.0305	1.4010	1.000	-6.2835	3.839
	5	1	.3538	1.4010	.999	-3.9009	4.224
		2	1.1655	1.4010	.920	-2.7048	5.035
		3	-2.3825	1.4010	.437	-6.2529	1.487
		4	.0305	1.4010	1.000	-3.8398	3.900
Glucose Tukey HSD	1	2	-6.2000	6.8507	.895	-25.125	12.725
		3	-33.9667*	6.8507	.000	-52.891	-15.042
		4	-8.7667	6.8507	.704	-27.691	10.158
		5	-13.4667	6.8507	.288	-32.391	5.458
	2	1	6.2000	6.8507	.895	-12.725	25.125
		3	-27.7667*	6.8507	.001	-46.691	-8.842
		4	-2.5667	6.8507	.996	-21.491	16.358
		5	-7.2667	6.8507	.826	-26.191	11.658
	3	1	33.9667*	6.8507	.000	15.042	52.891

		2	27.7667*	6.8507	.001	8.842	46.691
		4	25.2000*	6.8507	.003	6.275	44.125
		5	20.5000*	6.8507	.027	1.575	39.425
	4	1	8.7667	6.8507	.704	-10.158	27.691
		2	2.5667	6.8507	.996	-16.358	21.491
		3	-25.2000*	6.8507	.003	-44.125	-6.275
		5	-4.7000	6.8507	.959	-23.625	14.225
	5	1	13.4667	6.8507	.288	-5.458	32.391
		2	7.2667	6.8507	.826	-11.658	26.191
		3	-20.5000*	6.8507	.027	-39.425	-1.575
		4	4.7000	6.8507	.959	-14.225	23.625

*. The mean difference is significant at the 0.05 level.

Homogeneous Subsets

WBC

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	1	30	65.390	
	2	30	68.217	
	4	30	68.543	
	5	30	68.973	
	3	30		74.357
	Sig.		.215	1.000
Duncan[a]	1	30	65.390	
	2	30	68.217	
	4	30	68.543	
	5	30	68.973	
	3	30		74.357
	Sig.		.053	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

RBC

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1	30	1.7460		
	4	30	1.8783	1.8783	
	5	30	1.8960	1.8960	
	2	30	1.9783	1.9783	
	3	30		2.0690	
	Sig.		.069	.198	
Duncan[a]	1	30	1.7460		
	4	30	1.8783	1.8783	
	5	30	1.8960	1.8960	1.8960
	2	30		1.9783	1.9783
	3	30			2.0690
	Sig.		.110	.288	.064

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

HGB

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	1	30	7.9797		
	2	30	8.0400		
	4	30	8.2700	8.2700	
	5	30		8.5720	
	3	30			9.6393
	Sig.		.518	.478	1.000
Duncan[a]	1	30	7.9797		
	2	30	8.0400		
	4	30	8.2700	8.2700	
	5	30		8.5720	
	3	30			9.6393
	Sig.		.141	.104	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

HCT

	Treatment	N	Subset for alpha = 0.05
			1
Tukey HSD[a]	1	30	25.0337
	2	30	25.6933
	4	30	26.0000
	5	30	26.3233
	3	30	27.9067
	Sig.		.215
Duncan[a]	1	30	25.0337
	2	30	25.6933
	4	30	26.0000
	5	30	26.3233
	3	30	27.9067
	Sig.		.059

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

MCV

	Treatment	N	Subset for alpha = 0.05		
			1	2	3
Tukey HSD[a]	2	30	131.331		
	3	30	134.257		
	4	30	137.384	137.384	
	5	30	139.208	139.208	
	1	30		142.551	
	Sig.		.067	.413	
Duncan[a]	2	30	131.331		
	3	30	134.257	134.257	
	4	30	137.384	137.384	137.384
	5	30		139.208	139.208
	1	30			142.551
	Sig.		.055	.118	.102

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

MCH

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	2	30	42.289508393615000	
	4	30	44.880422858389580	44.880422858389580
	5	30	46.073834180674190	46.073834180674190
	1	30	46.199910838006744	46.199910838006744
	3	30		47.398797278313495
	Sig.		.108	.514
Duncan[a]	2	30	42.289508393615000	
	4	30	44.880422858389580	44.880422858389580
	5	30		46.073834180674190
	1	30		46.199910838006744
	3	30		47.398797278313495
	Sig.		.107	.154

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

MCHC

	Treatment	N	Subset for alpha = 0.05	
			1	2
Tukey HSD[a]	2	30	32.033482766855670	
	1	30	32.845174180483546	
	4	30	33.168443442258294	

	5	30	33.199017861974910	
	3	30	35.581616596764800	
	Sig.		.089	
Duncan[a]	2	30	32.033482766855670	
	1	30	32.845174180483546	32.845174180483546
	4	30	33.168443442258294	33.168443442258294
	5	30	33.199017861974910	33.199017861974910
	3	30		35.581616596764800
	Sig.		.456	.076

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.

Glucose

			Subset for alpha = 0.05	
	Treatment	N	1	2
Tukey HSD[a]	1	30	159.900	
	2	30	166.100	
	4	30	168.667	
	5	30	173.367	
	3	30		193.867
	Sig.		.288	1.000
Duncan[a]	1	30	159.900	
	2	30	166.100	
	4	30	168.667	
	5	30	173.367	
	3	30		193.867
	Sig.		.074	1.000

Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 30.000.