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Original Article

Influence of forzymedry© enzyme and protein levels on Nile tilapia, *Oreochromis niloticus* fry performance and histology.

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ABSTRACT

This study was performed to estimate the effect of protein, forzymedry© enzyme (cellulase, xylanase, B-glucanase, pectinase and galactosidase) and their interaction on the growth performance, body composition, some blood metabolites and histological statement of Nile tilapia, *Oreochromis niloticus* fry. A factorial trial (2 x4) two levels of dietary protein 20 and 25% (D20& D25) with four levels of forzymedry enzyem as feed additives (0, 25, 50 and 75 mg/kg diet). Results showed that, growth rate was not significantly differed by dietary protein but protein efficiency ratio (PER) was increased significantly (P<0.05) by increasing protein level. Increased dietary forzymedry enzyme level resulted in enhancing feed efficiency ratio, protein efficiency ratio, and survival rate significantly (P<0.05). The interacted effect influenced significantly (P<0.05) on all parameters of growth performance except for feed intake. And dietary 20% CP with adding 75mg/kg of enzyme had the best growth patamteres. The higher whole body protein of tested fish was obtained by 20% CP diet supplemented with adding 25 and 50 mg/ kg of Forzymedry© enzyme compared to other experimental diets. There were slightly differences among different interaction patterns of supplemental protein and enzyme for blood parameters as total protein, albumin, globulin and glucose. Also, there were no hazards effects on the histology statement with protein levels and/or forzymedry© enzyme. So, it can be use Forzymedry© enzyme with dietary lower total protein levels of 20 and 25% of Nile tilapia, *Oreochromis niloticus* fry.

Key words: protein levels, forzymedry© enzyme. Nile tilapia, growth performance, Histology
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1. INTRODUCTION

Different systems of fish production especially the intensive production of a particular species of fish relies upon many factors, one of which is a nutritionally complete, cost-effective, formulated feed. Protein is the most expensive ingredient in formulated feeds to meet the requirements of the cultured fish. Also, understanding the fish's protein requirement during the growth stage is necessary in fish culture management leading to enhanced several growth parameters (Abdel-Tawwab and Ahmad 2009).

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Protein requirements for optimum growth of the fish seem to be influenced by some factors such as temperature, salinity, fish age and size, etc. (Cowey, 1976). Tilapia is considered one the best species for culture because of its high tolerance to adverse environmental conditions, fast growth and easily stocking (Abdel-Aziz et al., 2021). The protein requirement of tilapia decreases with age and size. Higher dietary CP is required for fry and juvenile tilapia but lower protein levels for adult one (Winfree and Stickney 1981). On the other hand, a study was performed to evaluate the least–cost dietary protein level for four species of tilapia (*Oreochromus mossambicus, O. niloticus, O. aureus and Tilapia Zillii*) showed that, the dietary protein level from 34% to 36% provided maximum growth of young tilapia (1.5 g), but the most cost-effective protein level was 25% to 28% (De Silva et al., 1989). Currently, for minimize the production cost; commercial feeds for tilapia often contain lower protein levels (17–25 %) which were may be below recommended levels for ideal growth. At reduced protein levels, must be accompanied with saving other sources of energy to avoid the unprofitable effects of protein shortage on growth performance (El-Dakar et al., 2021). El-Sayed and Teshima (1992) found that, dietary protein requirements decreased with increasing fish weight and age. Both in fingerling and advanced juvenile of Nile tilapia, excess protein could not be utilized efficiently and might have been used for energy.

A number of studies have reported that fish can use carbohydrate as energy sources, for example, Nile tilapia and channel catfish (Wilson and Poe, 1985). These species digest over seventy percent of the gross energy in non-cooked starch. De Silva et al. (1991) reported that, dietary protein utilization may be improved by partially replacing dietary protein with lipid and/or carbohydrate to benefit from protein-sparing criterion. Supplementation of various enzyme mixtures in feed (FAO, 2006) is a potential way to improve nutritional value of the feedstuff. Several comparative studies on the exogenous digestive enzymes have been reported to understand the potential effects of several exogenous digestive enzymes in different fish species (Lemos and Tacon, 2016). Forzymedry© one of exogenous digestive enzymes which contains a cocktail of enzymes including cellulase, xylanase, B-glucanase, pectinase and galactosidase. So, this study was carried out to estimate the effect of protein, forzymedry© enzyme and their interaction on the growth performance, body composition, some blood metabolites and histological statement of Nile tilapia, *Oreochromis niloticus* fry.

2. MATERIALS AND METHODS

The current study was conducted at the Dept. of poultry production in the faculty of Agriculture, Minufiya University, Egypt. The laboratory analysis was performed in Dept. of fish nutrition in the faculty of Agriculture, Tanta University.

2.1. Experimental diets and design

The experimental diets formulation and chemical composition are provided in Table (1). The feed ingredients used in this study were obtained from local commercial suppliers including fish meal, soybean meal, wheat bran, rice bran, yellow corn, sunflower oil, mineral mixture and vitamins as a basal diet. Eight experimental diets were formulated as the following: four levels of forzymedry© enzyme (0, 25, 50, 75 mg/kg) was adding the diet 20 % CP and the same levels of enzyme were adding the diet 25% CP. Diets were prepared using a pelletizer unit and the ingredients were weighted and mixed thoroughly, followed by addition of corn oil and water. The wet mash was cold– pelleted into 35 mm diameter pellets. The resulting pellets were sun-dried and then stored at 5º C until fed or analyzed for chemical composition. Diets were formulated as dry pellets.

Table (1): Composition and proximate analysis of the experimental diets.

Ingredient, %	D1 (20%CP)	D2 (25%CP)
Fish meal, 60% CP	5	5
Soybean meal (SBM, 44% CP)	21	37
Yellow corn	34	31
Wheat bran	19	11
Rice bran [1]	13.5	8.5
Sunflower oil	4	4
Vitamin	0.5	0.5
Molasses	1.5	1.5
Di calcium phosphate	1	1
Minerals	0.5	0.5
Total	100	100
Chemical composition(% DM)		
Dry Matter	88.18	88.30
Crude protein	20.04	25.04
Ether extract	8.11	7.22
Crude fiber	3.91	4.48
Nitrogen Free Extract	55.15	51.11
Crude ash	6.08	6.24
Calculated energy value		
GE (Kcal/Kg) [2]	4226.5	4104.78
DE (Kcal/Kg) [3]	3169.95	3078.59
P/E (mg/Kcal) [4]	101.053	101.11

[1]*Vitamins and minerals mixture (mg or IU if mentioned kg ¯1diet): vitamin A, 8000 IU; vitamin D3, 4000 IU; vitamin E 50 IU; vitamin K3, 19IU;vitamin B2, 25mg; vitamin B3, 69mg; nicotinic acid, 125mg; thiamine, 10mg; folic acid, 7 mg; biotin, 7mg; vitamin B12,75mg; choline, 400mg and vitamin C, 200 mg. 300 mg I, 100mg Co, 100mg Si, 50000mg Zn, 70000mg Mn, 30000mg Fe, 4000 Cu, and CaCo3 even 1 KG.*

[2]*GE (Gross energy) was calculated according to NRC (1993) by using factors of 5.65, 9.45 and 4.22 Kcal per gram of protein, lipid and carbohydrate, respectively.*

[3]*DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher et al., (1983).*

[4]*P/E (protein energy ratio) = crude protein x 10000 / digestible energy, according to Hepher et al., (1983).*

2.2.Fish, facility and feeding trial

Nile tilapia, *Oreochromis niloticus* fingerlings were obtained from a local fish hatchery in Behara Governorate, Egypt. Fish were adapted to laboratory conditions for one week in fiber glass tank. At the beginning of the experiment, twenty four 80-L glass aquaria were stocked with 15 fish (1± 0.l g) for each one. The aquaria were continuously aerated using an air compressor. Overhead fluorescent lighting was set at 14:l0 (1ight: dark). The experimental diet was fed to experimental fish twice daily at 6% of body weight in the beginning and decreased to 5% by the end of the feeding trial during the period of 12 weeks feeding. Each group of fish was weighed at the beginning and every 2 weeks throughout the experimental period. The aquaria were cleaned daily and two thirds of the water replaced before feeding.

Water temperature and dissolved oxygen were measured every other day using an oxygen meter. Total ammonia and nitrite were measured twice weekly using a spectrophotometer. Total alkalinity and chloride were monitored twice weekly using the titration method and pH was monitored twice weekly using an electronic pH meter. During the 12-week feeding trial, the water-quality parameters averaged (± SD): water temperature, 25.6 ± 0.9 º C: dissolved oxygen, 6.5 ± 0.5 mg /l : total ammonia, 0.20 ± 0.14 mg /L : nitrite, 0.07 ± 0.05 mg /L : total alkalinity, 181 ± 45 mg/ L: chlorides, 575 ± 150 mg/ L : pH, 8.5 ± 0.2.

2.3.Sample collection and analysis

At the beginning of the study, 15 fish were sampled and frozen at -18 º C for analysis of whole body composition (AOAC, 1995). At the end of the feeding trial all fish were counted and weighed to calculate percent weight gain (PWG; [BW – initial BW] × 100/initial BW), feed efficiency ratio (FER; WG/ dry feed consumed), protein efficiency ratio (PER; WG/protein intake), specific growth rate (SGR; [ln final BW – ln initial BW] × 100/days), and survival ([no. of fish at the end of the experiment/no. of fish at the beginning of the experiment] × 100). Six fish from each tank were sampled for biochemical analysis. Also, fish were homogenized individually for whole body composition and frozen at-18 ºC for an approximate chemical analysis.

2.4.Histological analysis

For the histological examination, two randomly selected fish from each aquarium were sacrificed (n = 6 per treatment). The head and tail of each fish were cut off and the viscera were dissected and preserved in 10% neutral buffered formalin (Thermo

Fisher, Kalamazoo, MI) for 48 h. The following day, the viscera were washed with water several times and preserved in 75% ethyl alcohol for further processing. The liver, pancreas and kidney were separately dissected and examined. Tissues were routinely dehydrated in ethanol, equilibrated in xylene, and embedded in paraffin according to standard histological techniques. All tissues were longitudinally sectioned. Sections were cut to 5µm increments, mounted on glass slides and stained routinely with hematoxylin and eosin (H&E) stain for examination through the light electric microscope (Banchroft et al., 1996). After staining the sections were immersed in xylene and set in a Permount medium.

2.5.Statistical Analysis

Statistical analysis was performed with analysis of variance (ANOVA) using Statistical Analysis System (SAS, 2002) version 8.02 for Windows. The data (means ± SE, standard error) from each group were compared using Tukey's test (one-way ANOVA). Differences between treatments were considered significant when $P < 0.05$. Two-way ANOVA was also used to test the effects of dietary protein and enzyme Forzymedry© levels, as well as their interactions.

3. RESULTS AND DISCUSSION

3.1. Growth parameters

Table (2):- Effect of dietary protein levels, dietary Forzymedry© supplementation and their interaction on growth performance, feed utilization and survival rate of Nile tilapia fry.Data in table (2) showed that, protein level had no significant effect ($P>0.05$) on FW, WG, SGR, FI, FER and SR of experimental tilapia fry.

While, protein efficiency ratio (PER) was increased significantly ($P<0.05$) by increasing protein level. Also, Forzymedry© enzyme supplementation had no significant influence ($P>0.05$) on FW, WG, SGR and FI parameters. On the other hand, FER, PER and SR were increased significantly ($P<0.05$) by increasing dietary Forzymedry© enzyme level. Oppositely, in this study the interaction between protein and Forzymedry© enzyme levels affected significantly ($P<0.05$) on all estimated parameters of growth performance except for feed intake (FI). The best final weight was detected by diet contained 25% protein level with supplemented Forzymedry© enzyme at 50 mg/kg DM (6.27 g). Feeding diets with the lowest dietary protein and energy content may led to slower growth of fish but there were no significant differences among fish fed the other diets. These results demonstrate the tolerance of tilapia to a wide range of nutrient densities and the ability to tolerate a very low-protein diet. It has also been commented that, the relationship between dietary protein and energy in fish feeds should be considered for optimum fish performance. At inadequate energy levels, dietary protein may be used as an energy source, but, at adequate energy levels, dietary protein can be spared for anabolic functions (Cho and Kaushik 1985 and El-Sayed, 1987). In agreement with current results, Yan et al. (2012) explained that, in the 20 and 25% protein diets, energy retention significantly increased with increasing dietary energy which corresponds to reduce dietary carbohydrate content.

Table (2):- Effect of dietary protein levels, dietary Forzymedry© supplementation and their interaction on growth performance, feed utilization and survival rate of Nile tilapia fry.

Items	In wt[1]	FW[2]	WG[3]	SGR[4]	FI[5]	FER[6]	PER[7]	SR[8]
Effect of protein levels								
D1, 20% CP	0.94±0.01	5.88±0.29	525.00±31.86	2.16±0.07	10.69±0.43	0.46±0.05	1.84±0.06^{b}	82.25±3.00
D2, 25% CP	0.98±0.02	5.78±0.22	489.93±24.91	2.10±0.05	10.46±0.38	0.46±0.02	2.30±0.10^{a}	82.42±3.15
Effect of enzyme Forzymedry© supplementation levels (mg/kg diet).								
T1, Control	0.95±0.02	5.18±0.47	447.41±52.76	2.00±0.11	10.06±0.69	0.42±0.02^{a}	1.86±0.08^{b}	75.50±6.32^{b}
T2, 25	0.97±0.02	6.05±0.36	529.44±45.64	2.17±0.10	11.12±0.47	0.46±0.03^{ab}	2.06±0.19^{ab}	83.17±4.09^{ab}
T3, 50	0.97±0.03	5.90±0.30	511.90±32.46	2.15±0.07	10.64±0.53	0.46±0.02^{ab}	2.08±0.12^{ab}	83.67±2.75^{ab}
T4, 75	0.97±0.02	6.18±0.16	541.11±21.72	2.21±0.04	10.47±0.61	0.50±0.02^{a}	2.27±0.17^{a}	87.00±2.21^{a}
The interaction effect of protein and enzyme supplementation levels								
D1* T1	0.93±0.03	5.80±0.85^{ab}	520.37±87.86^{ab}	2.15±0.17^{ab}	10.95±1.19	0.44±0.03^{ab}	1.75±0.13^{d}	86.33±8.76^{a}
D1* T2	0.97±0.03	5.83±0.76^{ab}	508.15±93.15^{ab}	2.12±0.19^{ab}	10.94±0.72	0.44±0.04^{ab}	1.76±0.19^{d}	78.33±7.68^{ab}
D1* T3	0.93±0.03	5.63±0.61^{ab}	502.59±59.33^{ab}	2.13±0.12^{ab}	10.12±1.04	0.46±0.02^{ab}	1.85±0.11^{cd}	78.67±3.48^{ab}
D1* T4	0.93±0.03	6.23±0.27^{a}	568.89±32.27^{a}	2.26±0.05^{a}	10.75±0.84	0.50±0.01^{ab}	1.98±0.06^{bcd}	85.67±4.66^{a}
D2* T1	0.97±0.03	4.57±0.12^{b}	374.44±29.58^{b}	1.85±0.07^{b}	9.18±0.43	0.39±0.01^{b}	1.96±0.04^{bcd}	64.67±2.33^{b}
D2* T2	0.97±0.03	6.27±0.12^{a}	550.74±35.85^{ab}	2.23±0.06^{ab}	11.30±0.72	0.47±0.04^{ab}	2.37±0.21^{ab}	88.00±1.00^{a}
D2* T3	1.00±0.05	6.17±0.08^{ab}	521.21±40.76^{ab}	2.17±0.07^{ab}	11.17±0.14	0.46±0.01^{ab}	2.32±0.08^{abc}	88.67±0.88^{a}
D2* T4	1.00±0.01	6.13±0.23^{ab}	513.33±23.33^{ab}	2.16±0.04^{ab}	10.19±0.1.03	0.51±0.04^{a}	2.56±0.23^{a}	88.33±0.86^{a}

Means in the same column within each classification having different superscript letters were significantly different at P<0.05.

[1]In wt: initial mean weight (g), [2]FW: final mean weight (g), [3]WG: percent weight gain (%), [4]SGR: specific growth rate (% day^{-1}), [5]FI: feed intake (g dry diet fish^{-1} 60 days^{-1}), [6]FER: feed efficiency ratio, [7]PER: protein efficiency ratio, [8]SR: survival rates (%).

This suggests that carbohydrate may not have been utilized efficiently at the higher levels of inclusion with high protein levels. However, they showed that, fish fed on 20 and 30 % protein diets, manipulated minimal changes to energy retention values. The increase in energy retention was most pronounced in the 20 and 25 % protein series of diets. Generally, tilapia fed on diets containing at least 20% crude protein exhibited significantly greater body protein level than that fed the lower crude protein diets (EI-Dahhar et al., 2000). Balarin and Haller (1982) suggested that, normally 5–25 g Nile tilapia required protein levels between 25 and 35 % of the diet and 20 % protein do not satisfy the protein requirement of juvenile tilapia and lead to reduced growth of this fish. In this experiment, feed consumption was increased insignificantly (P < 0.05) as dietary crude protein decreased during the whole experimental period. The inclusion of exogenous enzymes in fish diets can be used to enhance the utilization of nutrients and reducing production costs. So, the greatest benefit of these enzymes (protease, α-amylase, cellulase, xylanase α-galactosidase, pectinase, phytase, endoglucanase, sucrase, and others) is improving the digestibility of plant feedstuffs in the diets. Adeoy et al.(2016) explained that, using diet supplemented with exogenous enzymes (phytase, protease and xylanase) for tilapia feeding improved significantly (P<0.05) estimated growth parameters FBW, SGR, FCR and PER compared to feeding tilapia the basal and probiotic supplemented diets. So, the growth improvement in current study may be due to enzyme inclusion which makes crude protein fractions more available to use as energy source for growth.

3.2. Body composition

Table (3):- Effect of dietary protein levels, dietary Forzymedry© supplementation and their interaction on whole body composition of Nile tilapia fry.

Items	Ash	Moisture	Lipids	Protein
Initial *	10.32±0.14	77.36±0.45	26.62±0.22	44.81±0.21
Effect of protein levels				
D1, 20% CP	13.13±0.26	74.98±0.25	25.22±0.16	40.75±0.44^{a}
D2, 25% CP	14.3±0.29	74.07±0.28	23.92±0.51	36.64±0.39^{b}
Effect of enzyme Forzymedry© supplementation levels (mg/kg diet).				
T1, 0 Control	14.85±0.77^{a}	75.62±0.57	25.71±0.45^{a}	36.45±1.10^{c}
T2, 25	13.98±0.49^{ab}	73.93±0.43	25.51±0.48^{a}	39.85±1.52^{a}
T3, 50	12.28±0.70 ^{b}	74.35±0.55	24.72±0.85^{b}	38.87±0.56^{ab}
T4, 70	13.75±0.50 ^{ab}	74.22±0.95	22.33±1.21c	39.62±1.62^{a}
The interaction effect of protein and enzyme supplementation levels				
D1* T1	13.16±0.21^{bc}	76.36±0.53^{a}	24.76±0.30^{b}	38.91±0.05^{c}
D1* T2	14.66±0.74^{ab}	74.03±0.64^{ab}	24.49±0.30^{b}	43.25±0.03^{a}
D1* T3	11.56±0.98^{c}	73.70±0.58^{ab}	26.59±0.26^{a}	37.60±0.05^{d}
D1* T4	13.13±0.63^{bc}	75.83±0.68^{ab}	25.03±0.31^{b}	43.25±0.03^{a}
D2* T1	16.53±0.24^{a}	74.86±0.88^{ab}	26.66±0.06^{a}	34.00±0.03^{g}
D2* T2	13.30±0.43^{bc}	73.83±0.72^{ab}	26.53±0.05^{a}	36.46±0.02^{e}
D2* T3	13.00±0.98^{bc}	75.00±0.85^{ab}	22.84±0.05^{c}	40.12±0.03^{b}
D2* T4	14.36±0.69^{b}	72.60±1.21^{b}	19.64±0.05^{d}	36.00±0.03^{f}

Means in the same column within each classification having different superscript letters were significantly different at P<0.05.

Table (3) explained that, both of protein level (20 and 25%) had no significant influence (P>0.05) on whole body composition parameters of experimental fish except for protein percentage. The higher (P<0.05) body protein percentage was obtained by fish fed diet contained 20% dietary protein (40.75%). On contrary, Forzymedry© enzyme supplementation affected significantly (P<0.05) on carcass parameters except for whole body lipids percentage which affected insignificantly (P>0.05) by enzyme supplementation. When the interaction between protein and Forzymedry© enzyme levels are found, the significant differences (P<0.05) were observed among different experimental diets. The higher whole body protein of tested fish was obtained by 20% CP diet supplemented with 25 and 50 mg/ kg DM of supplemented Forzymedry© enzyme (43.25%) compared to other experimental diets.

Mohsen et al. (2010) explained that, Nile tilapia fed the 25%-CP diet had lower content of protein and higher lipid content than fish fed the 35%- or 45%-CP diet, for all weight classes. Due to the high feed intake, nutrient utilization, and the high nutrient digestibility, the deposited nutrients increased. On the other hand, Farhangi and Carter (2007) observed that enzymes supplementation had no effect on carcass composition of rainbow trout. Similarly, Ng and Chong (2002) indicated

that supplementation of enzymes mixture contained Allzyme Vegpro (protease and cellulose), Ronozyme (glucanase and pectinase) and α-mannanase did not influence on the whole body components of red hybrid tilapia. Also, Lin et al. (2007) stated that tilapia fed diets supplemented with exogenous enzyme (neutral protease, b-glucanase and xylanase) show no significant difference in whole body moisture, protein, lipid and ash.

3.3. Blood parameters

Table (4):- Effect of dietary protein levels, dietary Forzymedry© supplementation and their interaction on blood parameters of Nile tilapia fry.

Items	ALT (U/dl)	AST (U/dl)	Albumin (g/dl)	Total protein (g/dl)	Glub (g/dl)	Glucose (mg/dl)
Effect of protein levels						
D1,20% CP	357.75±14.46^{b}	14.5±0.67^{b}	4.40±0.16	8.07±0.35	3.71±0.23^{b}	44.03±1.94^{a}
D2,25% CP	383.92±9.44^{a}	19±0.55^{a}	4.89±0.50	9.86±1.07	5.62±0.56^{a}	40.24±2.78^{b}
Effect of enzyme Forzymedry© supplementation levels (mg/kg diet).						
T1, 0 control	401±5.21^{a}	16.5±0.99	6.2±0.63^{a}	11.85±1.68^{a}	5.65±1.08	45.90±4.48^{a}
T2, 25	390.5±2.78^{a}	15.5±1.18	4.75±0.03^{b}	7.825±0.39^{b}	4.47±0.17	41.43±1.82^{b}
T3,50	362.83±16.33^{ab}	16.5±1.57	4.15±0.22^{bc}	8.1±0.54^{b}	3.95±0.34	42.95±4.72^{b}
T4, 75	329±22.90^{b}	18.5±1.26	3.5±0.10^{c}	8.1±0.85^{b}	4.60±0.85	38.25±1.32^{b}
The interaction effect of protein and enzyme supplementation levels						
D1* T1	402±1.15^{a}	15 ±1.15^{cd}	4.80±0.05^{b}	8.10 ±0.11^{e}	3.30±0.11^{d}	35.9±0.24^{f}
D1* T2	385 ±1.73^{a}	13 ±0.57^{d}	4.72±0.04^{b}	8.70 ±0.11^{d}	4.15±0.05^{c}	45.5±0.23^{c}
D1* T3	365 ±2.30^{a}	14 ±2.20^{d}	4.60±0.17^{b}	9.30 ±0.02^{c}	4.70±0.05^{c}	53.5±0.05^{b}
D1* T4	288 ±6.53^{b}	16±0.57^{bcd}	3.50±0.02^{c}	6.20±0.08^{g}	2.70±0.11^{d}	41.2±0.17^{d}
D2* T1	400±11.54^{a}	18±1.15^{abc}	7.60±0.11^{a}	15.60±0.17^{a}	8.00±0.57^{a}	55.9±0.40^{a}
D2* T2	396±2.30^{a}	18±0.57^{abc}	4.80±0.01^{b}	6.95±0.02^{f}	4.80±0.17^{c}	37.37±0.02^{e}
D2* T3	394±15.37^{a}	19±0.86^{ab}	3.70±0.09^{c}	6.90±0.10^{f}	3.20±0.01^{d}	32.4±0.28^{g}
D2* T4	379±3.46^{a}	21±1.15^{a}	3.50±0.23^{c}	10.00±0.17^{b}	6.50± 0.11^{b}	35.3±0.12^{f}

Means in the same column within each classification having different superscript letters were significantly different at P<0.05.

The present data in table (4**)** pointed that, increasing dietary protein from 20 to 25% increased significantly (P<0.05) liver enzymes contained ALT and AST concentration beside blood globulin level. While, blood glucose was decreased significantly (P<0.05) by increasing dietary protein level. On other hand, blood total protein and albumin levels were not differed significantly (P>0.05) by supplemental protein. Furthermore, Forzymedry© enzyme supplementation influenced significantly (P<0.05) on blood concentration of ALT enzyme, total protein, albumin and glucose. Oppositely, no significant differences (P>0.05) were observed with supplemental enzyme levels for blood concentration of globulin and

glucose. Additionally, the current results explained that, the interaction between dietary protein and enzyme had significant effect on all estimated blood parameters in this experiment. In general the liver activity was increased by increasing both of dietary protein and Forzymedry© enzyme supplementation. There were slight differences among different interaction patterns of supplemental protein and enzyme for blood concentration parameters as total protein, albumin, globulin and glucose.

Biochemical parameters are considered as useful procedures for checking the quality of fish health and metabolic process and nutrient absorption that affecting fish (Hoseinifar et al., 2011). Khalafalla and El-hais (2013) found insignificant influence ($p>0.05$) of exogenous enzyme addition Nutrasexylam© enzyme contained a mixture of β-xylanase and α -amylase was obtained with parameters of serum protein (Total protein, albumin and globulin and enzyme addition at 0.10 g/kg had the best value of blood total protein and globulin.

3.4. Histology study

No histological changes were noted in liver or pancreas and kidney (Fig. 1-4). Liver of fish showing normal histological structure, the hepatocytes (h) in the parenchyma as all as portal vein (PV) in parallaxes in all groups. The pancreatic histopathological picture did not show any changes in samples from all dietary groups (Fig. 1, 2). Kidney of fish in all groups showing normal histological structure of glomeruli (g) and tubules (t) (Fig. 3, 4). No significant differences between fish fed the control and the other diets were found ($P > 0.05$).

Figure captions

Fig.1. Liver and pancreas histology of nile tilapia fed different diets (A, basal diet DL1) without any supplementation and contain low protein level (20% CP), followed by three diets supplemented with foryzmedry© enzyme at 25, 50, 75 mg/kg diet (B, C, D, respectively) showing normal histological structure, the hepatocytes (h) in the parenchyma as all as portal vein (pv) in parallaxes, (H&E staining); scale bars = 40 µm.

Fig.2. Liver and pancreas histology of nile tilapia fed different diets (E, basal diet DH1) without any supplementation and contain a high protein level (25% CP), followed by three diets supplemented with foryzmedry© enzyme at 25, 50, 75 mg/kg diet (F, G, H, respectively) showing normal histological structure, the hepatocytes (h) in the parenchyma as all as portal vein (pv) in parallaxes, (H&E staining); scale bars = 40 µm.

Fig. 3. Kidney histology of nile tilapia fed different diets basal diet DL1) without any supplementation and contain low protein level (20% CP), followed by three diets supplemented with foryzmedry© enzyme at 25, 50, 75 mg/kg diet (B, C, D, respectively) showing normal histological structure of glomeruli (g) and tubules (t), (H&E staining); scale bars = 40 µm.

Fig.4. Kidney histology of nile tilapia fed different diets (E, basal diet DH1) without any supplementation and contain a high protein level (25% CP), followed by three diets supplemented with foryzmedry© enzyme at 25, 50, 75 mg/kg diet (F, G, H, respectively) showing normal histological

structure of glomeruli (g) and tubules (t), (H&E staining); scale bars = 40 µm. Figure(1)

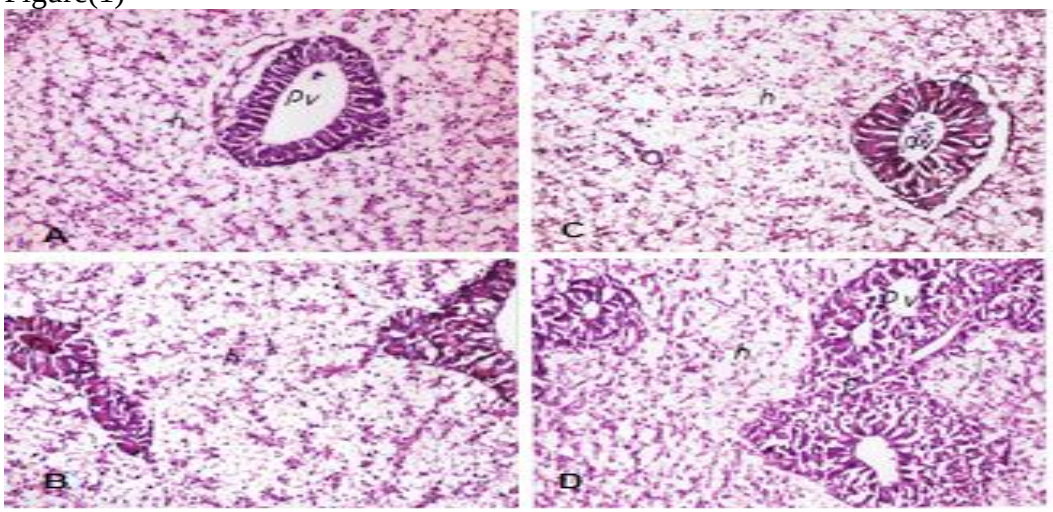


Figure (2)

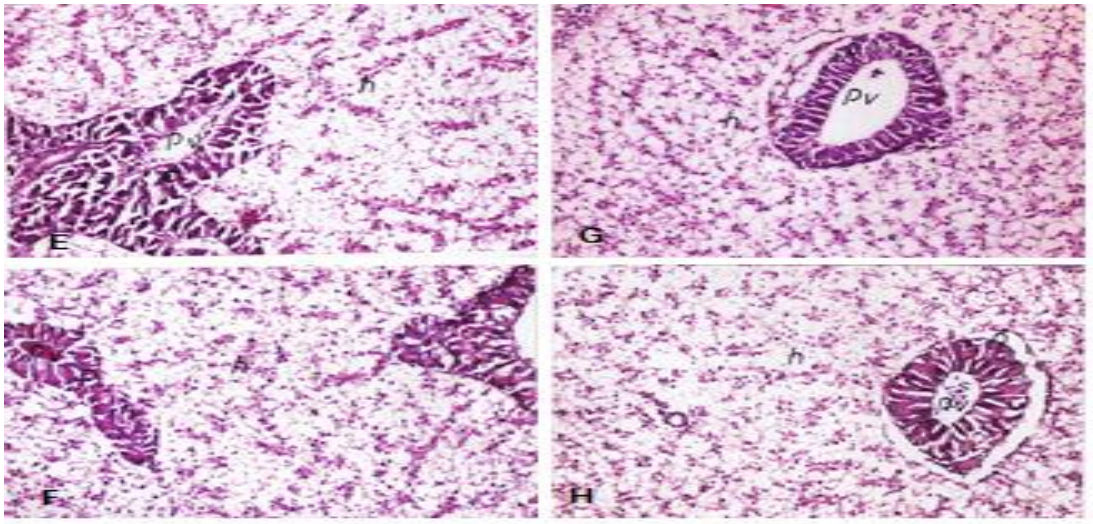
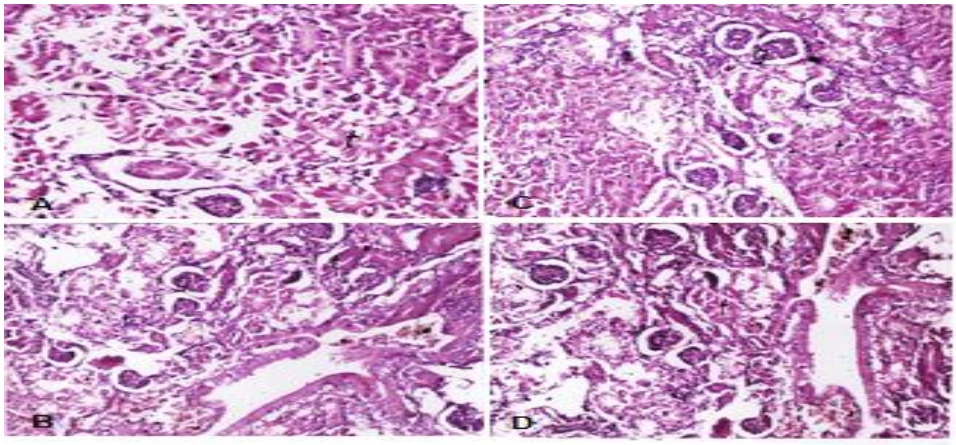
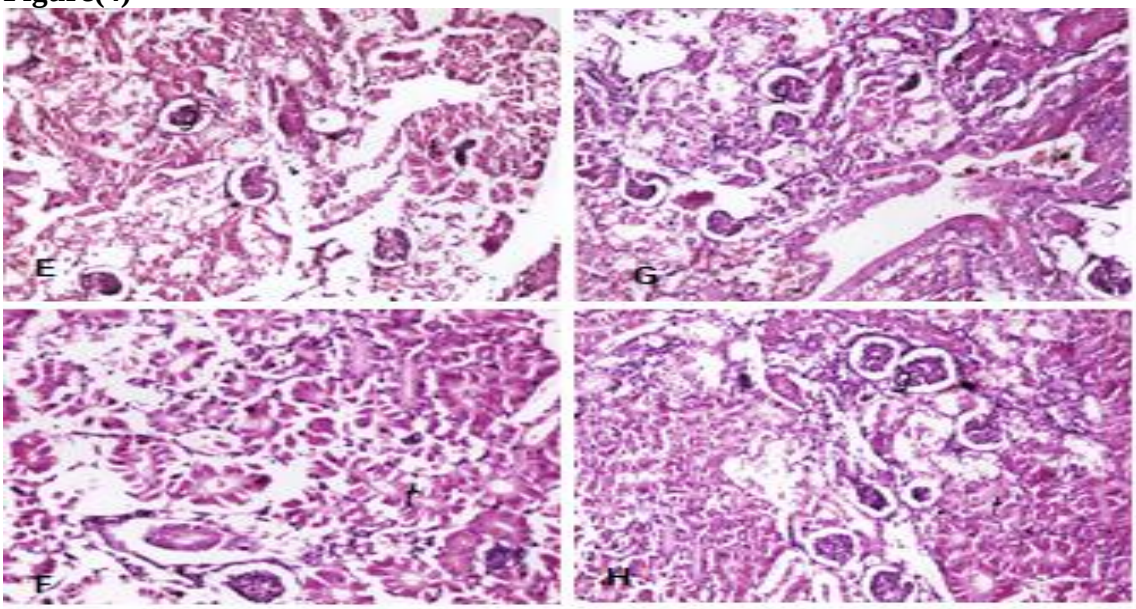


Figure (3)



Figure(4)



4. CONCLUSION

Forzymedry© enzyme supplementation can improve performance, growth without hazard effects on heamatological, histological and biochemical parameters of tilapia fry. So, it can be use Forzymedry© enzyme with dietary lower total protein levels 20 and 25% of Nile tilapia, *Oreochromis niloticus* fry.

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