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Full Length Research Paper

Effect of Turmeric (*Curcuma longa*) Supplementation on Growth Performance, Feed Utilization, and Resistance of Nile tilapia (*Oreochromis niloticus*) to *Pseudomonas fluorescens* Challenge

[*1]Manal M. A. Mahmoud, [2]Maather M.M. El-Lamie, [3]Amina A. Dessouki and [4]Mohamed S. Yusuf

[1,4]Department of Nutrition and Clinical Nutrition, Suez Canal University, Ismailia, Egypt
[2]Fish Diseases and Management, Faculty of Veterinary Medicine,Suez Canal University, Ismailia, Egypt
[3]Department of Pathology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

*Corresponding Author's E-mail: dr.manalah@gmail.com

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180 Nile tilapia fish were used in 3 months growth trial to study the effect of turmeric on growing tilapia. Fish were divided into three treatment groups. The first group T1 was given the basal diet without any supplementation of turmeric and served as the control group. The second groupT2 was given diet supplemented by 0.25% turmeric powder. The third groupT3 was given diet supplemented by 0.50% turmeric powder. At the end of the growth trial, fish were challenged with pathogenic *Pseudomonas fluorescence.* Turmeric supplementation non-significantly improved growth performance. There was a trend of higher values with increasing the turmeric supplementation level, and significant improvement in feed consumption in T3 compared toT1 and T2. Fish body composition was affected by turmeric supplementation. Crude protein content was significantly increased in T3 compared to T1. Ether extract content was significantly decreased with increasing the turmeric supplementation level as T1 was the highest in ether extract content and T3 was the lowest, this was significantly reflected on the gross energy (GE) content of the fish. The clinical signs in the challenged fishes were observed at the second day post injection. Fish showed loss of balance, excessive mucus secretions on skin and gills, ascites with slightly protruded reddish vent and hemorrhages all over the body surface, frayed and torn tail and fins, with no mortalities in the 0.50% turmeric supplemented group. We concluded that 0.50% turmeric supplementation may improve growth performance and significantly protect fish against *P. fluorescens.*

Keywords: Turmeric, growth performance, Nile tilapia, *Pseudomonas fluorescens* challenge

INTRODUCTION

Aquaculture is still a growing industry for having a high protein food. Different studies were conducted to minimize fish feed costs by using medicinal plants "back to nature" as feed additives instead of using synthetic drugs of serious side effects (Flück *et al.,* 1976). Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae (Chan *et al.,* 2009). It is native in southeast India. *Curcumin*, the active ingredient from the spice turmeric is a potent antioxidant (El-Bahr *et al.*, 2007; and Salama and El-Bahr, 2007) and has hepatoprotective properties (Pal *et al*., 2001). It is also strongly alleged that turmeric can improve digestion and nutrient metabolism. The latter beneficial effects of turmeric are related to atsiri oil and curcumin content in turmeric (Al-Sultan and Gameel, 2004). Atsiri oil can improve the function of digestive tracts (small intestine) and stimulate the production of digestive enzymes resulting in improved digestion and increased nutrients metabolism (Darwis *et al*., 1991).

Bacterial infections are the main causes of deaths in aquaculture (Abutbul *et al*., 2005). Aeromonad, Pseudomonad and Edwardsiella are naturally present in aquaculture and acting to cause diseases for fish (Badran and Eissa, 1991; Banu, 1996; Islam, 1996;

Table 1: Ingredient and composition of experimental diets

Ingredients %	T1	T2	T3
Fish meal (60.05)[1]	20.00	20.00	20.00
Soya bean meal (45%)[1]	23.86	23.87	23.98
Corn gluten (62) [1]	8.00	8.00	8.00
Ground yellow corn (8.5) [1]	16.84	17.07	17.31
Wheat flour (11.43) [1]	25.30	24.81	24.21
Vegetable oil	3.00	3.00	3.00
Minerals and vitamins premix[2]	3.00	3.00	3.00
Vitamin C (mg/kg diet)	50	50	50
Turmeric powder	-	0.25	0.50
Calculated composition			
Crude protein %	32	32	32
DE (kcal/kg)	3000	3000	2760
P/E ratio (mg protein/kilocalories DE	106.7	106.7	106.7

[1] Determined according to AOAC, 1995.
[2] Each 3 kg contain the following vitamins and minerals:
Vit. A 15 mIU, vit. D_3 2 mIU, vit. E 1000mg, vit. k_3 1000mg, vit. B_1 1000mg, vit. B_2 5000mg, vit. B_6 1500mg, vit. B_{12} 10mg, biotin 50mg, pantothinic acid 10000mg, nicotinic acid 30000mg, folic acid 1000mg, manganese 60000mg, zinc 50000mg, iron 30000mg, copper 4000mg, iodine 300mg, selenium 100mg, cobalt 100mg, carrier(CaCO3) to 3kg. (Golden premix- Selim Pharm Elasher, Egypt).

Khalil *et al*., 2010).Protecting fishes against diseases can be obtained by two methods. The first is by increasing the immunity of fish to fight the invasion of pathogens, and the second is through medication (Stephen *et al*., 2006). Drug-resistant strains are developed through medication with antibiotics (Harikrishnan and Balasundaram, 2011).Also, antibiotics results in presence of antibiotic residues in fish consumed by human (FAO, 2002). The wide range of fish pathogens in aquaculture also limits vaccines' effectiveness (Jaya kumari and Sahoo, 2006). Immunostimulants used instead of vaccination and medication in controlling fish diseases because they can enhance the non-specific immune response (Sakai, 1999). Also, immunostimulants help fishes under stress to enhance their growths and increase their survival rates **(**Heo *et al*., 2004). Addition of herbal extracts or their products in diet or by injection route enhance the innate and adaptive immune response of different freshwater fish against different diseases (Harikrishnan *et al*., 2011). Turmeric feeding may elevate the non-specific immune system and give long term of protection (Sahu *et al.*, 2008). Sivagurunathan *et al*., (2011) enhanced the nonspecific immune response of *Cirrhinus mrigala* by feeding diet containing *Zingiber officinale* and *Curcuma longa* when exposed to *Pseudomonas aeruginosa.*

The present study was conducted to determine the effects of two levels of turmeric on the growth performance, feed utilization, and resistance of Nile tilapia to Pseudomonas fluorescens infection.

MATERIAL AND METHODS

Experimental procedure

I- Growth performance trial

Before the start of the trial all Nile tilapia (*Oreochromis niloticus*) were reared in two 500 L tanks filled with aerated fresh water for 2 weeks to acclimate to the experimental conditions. Fish were fed on the control pelleted diet twice daily. At the start of the experiment, the fish were fasted for 24 h then weighed individually. Ten fish were frozen for determination of whole body proximate composition. Twenty fish (9.29 g average initial body weight) / glass aquarium (80 x 40 x 45 cm) were used with three aquaria per treatment (a total of 180 Nile tilapia fish). Fish were fed at a rate of 3% of their body weight twice daily at 9 am and 3 pm, 7 days a week. Each aquarium was supplied with low-pressure automatic aerator. The water temperature was maintained at 27±1ºC, pH 7.21 ± 0.12. The photoperiod used was a 14 h light/10 h dark cycle. Every other day, all the aquaria were cleaned before the morning feeding by siphoning the wastes accumulated on the bottom. About two-thirds of the water was replaced by aged aerated water from the storage tanks. Dead fish were daily recorded and removed. Fish were bulk weighed every two weeks and the amount fed was adjusted accordingly. At the end of the three months experiment, four fish from each aquarium were individually weighed,

sacrificed. The viscera were removed; the liver and the carcass were weighed individually to calculate the dressing percentage and the hepatosomatic index (HSI). Another two fish were sampled randomly from each replicate group, sacrificed and frozen for determination of whole body proximate composition.

Diets preparation

All the experimental diets were formulated to supply 32% crude protein and 3000 kcal digestible energy/kg diet according to NRC, 1993(Table 1). The first group T1 was given diet without any supplementation of turmeric powder and served as the control group. The second groupT2 was given diet supplemented by 0.25% turmeric powder. The third groupT3 was given diet supplemented by 0.50% turmeric powder. The ingredients were finely ground and mixed. About 400 ml of cold water/kg diet was added to obtain stiff dough. The obtained dough was passed through (2 mm) die of a meat mincer, pelleted then air dried by electric fan at room temperature for 24 h. The pellets were packed in plastic bags and refrigerated at 4 ºC until use (El-Ashram and El-Boshy, 2008).

Studied parameters

Fish performance was assessed by calculating body weight gain, specific growth rate, feed efficiency ratio, protein efficiency ratio, apparent energy utilization, survival rate percentage, dressing percentage and hepatosomatic index according to Kim *et al*., (2013) and Abdel-Tawwab and Ahmad (2009).

II-The challenge trial

At the end of the growth performance trial, fish continued to receive the same assigned diets. A well identified virulent strain of *Pseudomonas fluorescens* was kindly obtained from Department of Fish Diseases, Animal Health Research Institute, Dokki, Giza, Egypt. Before challenge, all fish were clinically healthy; there was no evidence of *Pseudomonas florescens* infection or any deaths due to *Pseudomonas florescens.* The 3 replicates from each treatment group (T1, T2 and T3) were divided into 2 subgroups (each had 6 fishes), kept in well prepared glass aquaria with aerated chlorine-free tap water, water temperature was adjusted at 27 C^{o}±1, pH was 7.3± 0.12, dissolved oxygen was 4.2-6.4 mg/l, nitrite (NO_2) was 0.04 -0.15 mg/l and unionized ammonia (NH_3) was 0.07-0.13 mg/l. All of these water quality parameters were within the acceptable range for fish growth (Boyd, 1984). The first six fishes from each replicate were intrapretonealy (I/P) injected with 0.3 ml sterile saline as negative control for the challenged control subgroups. The other six (T1+,T2+ and T3+) were challenged intraperitonealy (I/P) with 0.3 ml of sterile saline containing ($1.5X10^8$ CFU/ml) of *Pseudomonas fluorescens* according to Lucky (1977). The challenged fish were kept under observation for 15 days. Clinical signs, mortalities, postmortem lesions were recorded. All dead fish were examined to determine the aetiological agent. The relative level of protection (RLP) among the challenged fish was determined according to Amend (1981) using the following equation: RLP % = 1 – (percent of mortality in treated group/ percent of mortality in control group) × 100.

Histopathological examination

Specimens from liver, intestine and spleen of experimental fish were fixed in 10% neutral buffered formalin. Paraffin sections (5 micron thick) were prepared and stained with haematoxylin and eosin (HandE) and examined under light microscope according to Bancroft and Gamble (2007).

Statistical analysis

The obtained data were subjected to one-way ANOVA to evaluate the effect of turmeric supplement. Differences among means were tested at the 5% probability level using Duncan Multiple Range test. All the statistical analyses were done using SPSS program version 16 (SPSS, Richmond, VA, USA) as described by Dytham (1999).

RESULTS

Turmeric supplementation non-significantly improved the measured growth and feed utilization parameters. There was a trend of higher values with increasing the turmeric supplementation level, and significant improvement in feed consumption in T3 compared toT1 and T2 (Table 2). Fish body composition was affected by turmeric supplementation (Table 3). Crude protein content was significantly increased in T3 compared to T1. Ether extract content was significantly decreased with increasing the turmeric supplementation level as T1 was the highest in ether extract content and T3 was the lowest, this was reflected on the gross energy (GE) content of the fish. GE was decreased by increasing the turmeric level, T3 significantly had lower value compared to T1.

The clinical signs in the challenged fishes were observed at the second day post injection. Fish showed loss of balance, excessive mucus secretions on skin and gills (figure 1a), ascites with slightly protruded reddish vent (figure 1b) and hemorrhages all over the body surface, frayed and torn tail and fins (figure 1c, d). The postmortem lesions were congestion and enlargement of

Table 2: Growth performance of Nile tilapia fed different experimental diets

Parameters	T1	T2	T3
Initial weight (g/fish)	9.30	9.29	9.30
Final weight (g/fish)	41.99±1.27	44.43±2.95	46.06±0.28
Body weight gain (g/fish)[1]	32.69±1.27	35.14±2.95	36.76±0.28
Specific growth rate (SGR,%BW/day)[2]	0.78±0.02	0.81±0.03	0.82±0.00
Feed consumed (g/fish)[3]	47.94[b]±0.74	48.11[b]±1.25	52.48[a]±0.24
Feed Efficiency ratio (FER)[4]	0.68±0.02	0.73±0.04	0.70±0.01
Protein Efficiency ratio (PER)[5]	0.65±0.12	0.67±0.03	0.69±0.00
Apparent energy utilization (AEU)%[6]	23.32±1.63	23.66±1.56	20.85±0.64
Survival rate %[7]	90.00±2.88	93.33±3.33	90.00±2.88
Dressing percentage[8]	84.25±0.67	84.52±0.53	84.65±0.55
Hepatosomatic index (HSI)[9]	2.82±0.32	2.94±0.16	3.29±0.24

[a-b] Means in the same row with different superscripts are significantly different (p≤ 0.05); values are presented as means ± SE.

1- Body weight gain (WG) = FBW- IBW (g/fish)
2- Specific growth rate (SGR, %body weight/day) = 100x [log FBW (g) - log IBW (g)]/ time (days)
3- Feed consumed (g/ fish) =total feed consumed over 83 days (g)/ number of fish.
4- Feed efficiency ratio (FER) (g/g) = WG (g)/feed consumed (g)).
5- Protein efficiency ratio (PER) (g/g) = WG (g)/ protein consumed (g).
6- Apparent energy utilization (AEU) (%) = energy gain (MJ/fish) x 100/energy intake (MJ/fish).
7- Survival rate percentage = 100x (total number of fish at the end of the experiment/ total number of fish at the start of the experiment).
8- Dressing percentage = 100x (dressed carcass weight (g)/live weight (g).
9- Hepatosomatic index (HSI) (%) = 100x (liver weight (g)/body weight (g).

Table 3: Body composition of Nile tilapia fed different experimental diets (on % wet basis) [1]

Parameters	T1	T2	T3
Moisture	70.69±0.74	70.04±42	69.86±0.24
Crude protein	15.71[b]±0.41	16.41[ab]±0.19	16.78[a]±0.12
Ether extract	5.12[a]±0.31	4.14[b]±0.13	3.00[c]±0.31
Ash	3.67±0.14	4.13±.30	3.97±0.28
Gross Energy ($MJ.Kg^{-1}$) [2]	5.74[a]±0.21	5.52[ab]±0.07	5.15[b]±0.14

1. Composition of the fish killed at the beginning of the experiment (moisture, 74.65 %; crude protein, 14.41 %; ether extract, 3.52 %; ash, 4.27 % and gross energy, 4.80 $MJ.Kg^{-1}$). Determined according to AOAC, 1995.
2. The gross energy content of fish was calculated from the fat and protein contents, using the equivalents of 39.54 $MJ.kg^{-1}$ for fat, and 23.64 $MJ.kg^{-1}$ for crude protein (Kleiber 1975).

[a-c] Means in the same row with different superscripts are significantly different (p≤ 0.05); values are presented as means ± SE.

Table 4: Results of challenge with *Pseudomonas fluorescens* on mortality% and RLP%:

challenged fish subgroups	Level of supplemented turmeric (%)	No. of challenged fish	No. of dead fish	Mortality%	RLP%
(T1+)	0	18	6	33.33	0
(T2+)	0.25	18	2	11.11	66.67
(T3+)	0.50	18	0	0	100

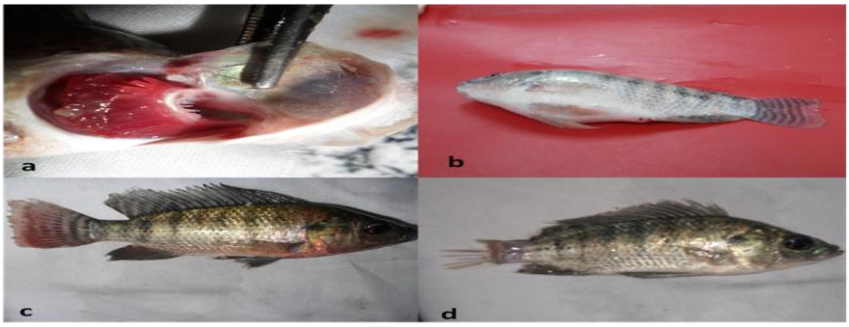

Figure (1)*: Oreochromis niloticus* (a) showing excessive mucus secretion on gills, (b) showing ascitis with slightly protruded reddish vent and (c) and (d) hemorrhagic, frayed and torn fins and tail.

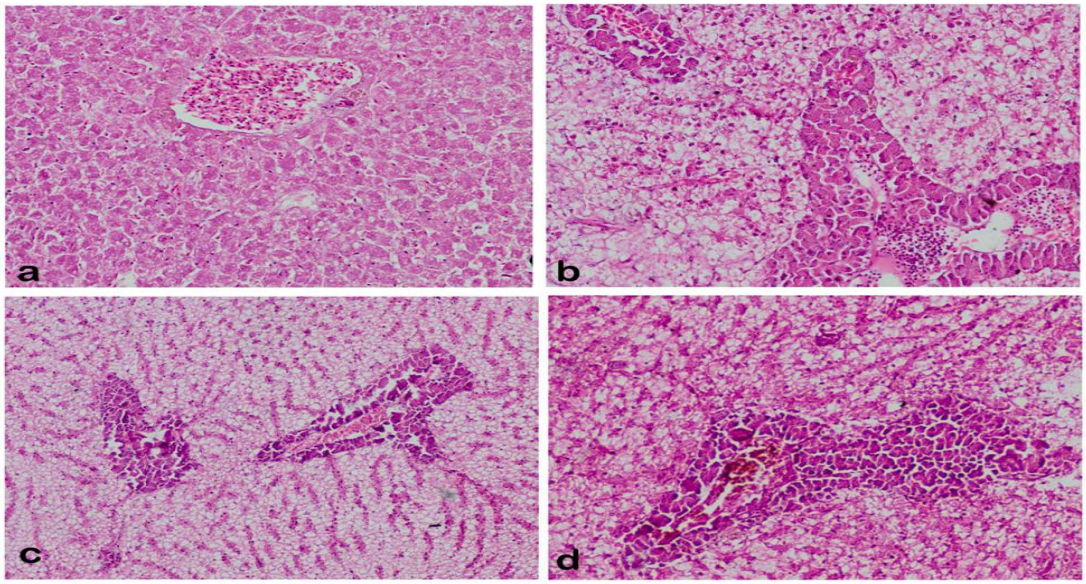

Figure (2): Liver (a) showing normal structure, (b) showing vacuolar degeneration of hepatocyts, leukocytic infiltration of hepatopancreas. (c) mild degeneration of hepatocytes (d) mild degeneration of hepatocyts. H and E. x400

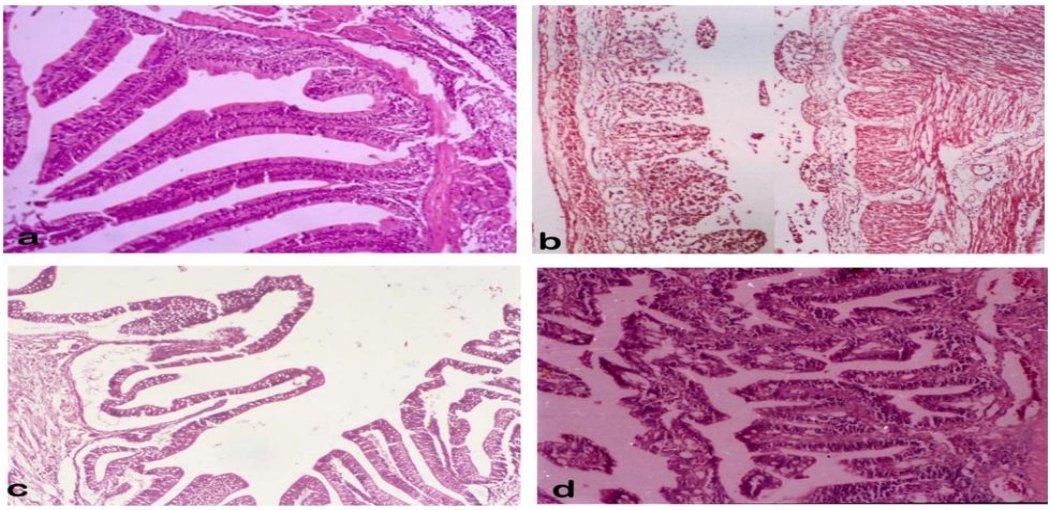

Figure (3): Intestine (a) showing normal architecture of intestinal villi . (b) showing severe degeneration of epithelial cells lining the intestinal villi along with leukocytic infiltration .(c) showing slight hyperplasia of epithelial lining the intestine villi .(d) showing slight hyperplasia of epithelial lining the intestine villi . H and E. x 200

internal organs. Mortalities were shown only in the challenged subgroups (T1+ and T2+).

Liver of T1, T2 and T3 showed normal hepatocytes with normal arrangement of hepatic cords. The central veins and hepatopancrease also showed nomal histology (figure 2a). Group T1+ showed congestion of central veins and hepatic sinusoids, hyperplasia of pancreatic acini which was infiltrated by mononuclear cells. Diffuse vacuolar degeneration of the hepatocytes was also observed (figure 2b). Group T2+ **s**howed vacuolation of hepatocytes and mild congestion of central veins (figure 2c). Group T3+ showed mild degree of degeneration along with activation of melano-macrophage centers and Kupffer cells (figure 2d).

Intestine: group T1 showed normal architecture of intestinal villi (figure 3 a). Groups T2 and T3 were similar to group T1. Group T1**+** showed severe degeneration of epithelial cells lining the intestinal villi along with leukocytic infiltration (figure 3b). Group T2**+** had slight hyperplasia of epithelial lining the intestine villi (figure 3c). Group T3**+** showed slight hyperplasia of epithelial lining the intestine villi and mild vacuolar degeneration (figure 3d).

Spleen**:** group T1 showed normal architecture of both red and white pulps (figure 4 a). Groups T2 and T3 were similar to group T1. Group T1**+** showed depletion of

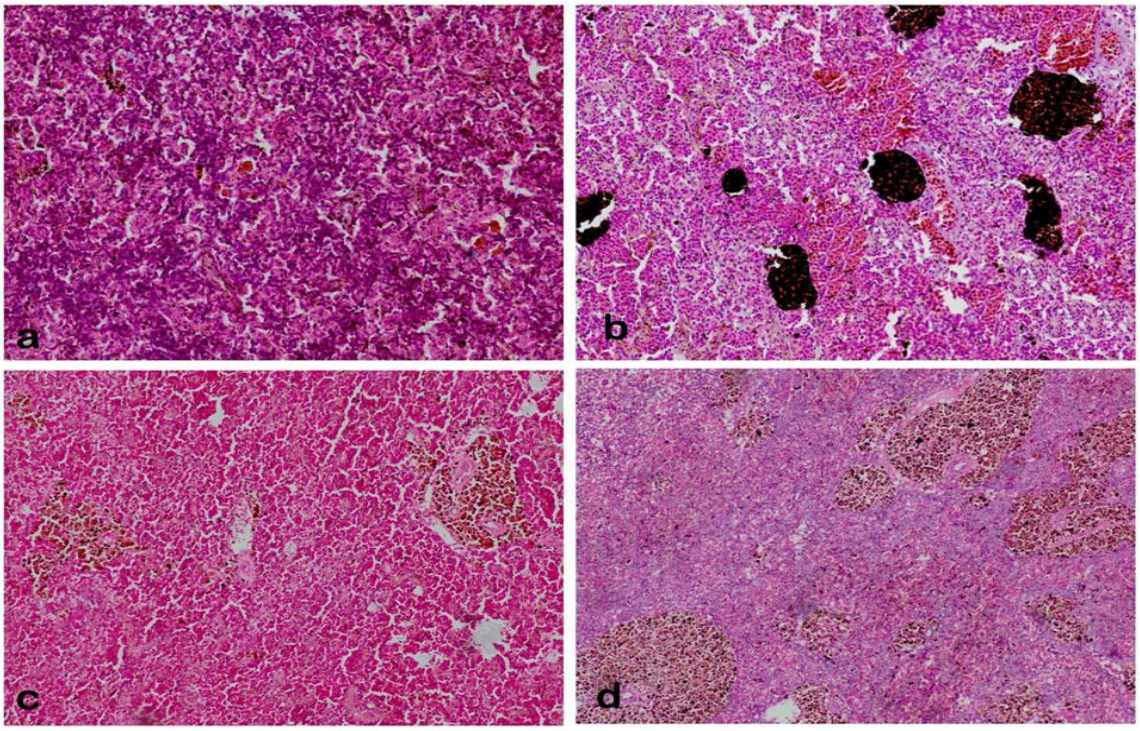

Figure (4): spleen (a) showing normal structure, (b) showing necrosis of lymphocytes, congestion of sinusoids and hyperplasia of melanomacrophage centers (c) mild depletion and activation of melanomacrophge center. (d) hyperplasia of lymphocytes and activation of melanomacrophge center . H and E. x 200.

lymphocytes and activation of melanomacrophage center and congestion of sinusoides (figure 4b). Group T2**+** had slight hyperplasia of lymphocytes and activation of melanomacrophage (figure 4c). Group T3**+** had slight hyperplasia of lymphocytes and activation of melanomacrophage (figure 4d).

DISCUSSION

Medicinal plants have received increasing attention as spices for human and additive in diets for animals. However, only few studies have been done on the use of feed additives in fish nutrition (El-Bahr and Saad, 2008; Lawhavinit *et al*., 2011). The enhanced growth response indicated by turmeric supplementation in this study may be due to improved feed consumption, improved feed utilization, which is an indication of increased nutrient digestibility and antioxidant activity of Turmeric (Osawa *et al.,* 1995) that stimulates protein synthesis by enzymatic system. This is in accordance to Pransin,(2006) who reported that goldfish fed turmeric supplemented diets, had highest acid protease, alkaline protease and lipase activity, enhanced growth rate and yellow pigmentation. Also, Rojtinnakorn *et al*., (2012) results showed that all turmeric extract fed fish had significant higher specific activities of digestive enzymes and indicated that growth rate will be enhanced in follow up.

No study is available on turmeric effects on the whole body composition of fish. In the present study, fish body composition was affected by turmeric supplementation. Crude protein content was significantly improved. Ether extract content was significantly decreased with increasing the turmeric supplementation level, which leads to GE reduction by increasing the turmeric level. This is in accordance with (Nouzarian *et al.,* 2011 and Hussin, 2013) who reported a significant decrease in abdominal fat pad in chickens fed the turmeric supplemented diets. Also, Daneshyar *et al.,* (2011) concluded that supplementation of turmeric powder in broiler chickens diets can decrease the concentrations of saturated fatty acids and triglycerides in thigh meat and improve the meat quality as a result. Hussin (2013) observed significantly increased crude protein % in breast meat and significantly decreased ether extract % in thigh meat in broilers supplemented with turmeric powder 7 g TP/ kg diet than (5, 9 g TP/ kg diet) and non-supplemented group. The cause of the increased crude protein % in breast meat and the increased breast and thigh weight is the Curcumin that stimulated the digestive system in poultry, by improving the utilization of digestive products (Hernandez *et al.,* 2004). This is consistent with Pransin,(2006) and Rojtinnakorn *et al.,*

(2012), who reported that fish showed significant higher specific activities of digestive enzymes due to turmeric supplementation.

The disease challenge is an in vitro technique which give the chance to determine the performance and immunity of fish when exposed to xenobiotic (bacteria) present in their natural habitats (AraKoosh *et al.,* 2005). The challenge infection using *P. fluorescens* revealed low mortality percentage in T2+ receiving 0.25% turmeric in diets and no mortality in T3+ receiving 0.50% turmeric. A high level of protection (RLP) was obtained in T3+ (100%) than in T2+ (66.67%) with no protection in the challenged control subgroups T1+. These results were in agreement with Amany Diab *et al.,* (2014) who recorded that the mortality rate in *Oreochromis niloticus* curcumin-treated groups (with 1% and 2% curcumin in diet) after challenging with *Pseudomonas fluorescens* were decreased than in control group. They concluded that curcumin in diets of *Oreochromis niloticus* improve growth performance and immunity which increases the resistance of challenged fish to *Pseudomonas fluorescens*. Also, Sahu *et al.*, (2008) detected 100% and 89% survivability in *Labeo rohita* groups fed with 5.0 and 1.0 g tumeric/kg feed for 60 days, respectively after challenge with *Aeromonas hydrophila.*

The histopathological examination in the present study illustrated the improved performance and immunity as shown in spleen where the groups treated with curcumin showed hyperplasia of lymphoid follicles and melanomacrophage centers. The histopathology of liver and intestine that treated with cucumin showed mild degeneration to almost normal hepatic cords and intestinal villi respectively. This desired effect of turmeric is attributed to its potent antioxidant (El-Bahr *et al.,* 2007; and Salama and El-Bahr, 2007) and its hepatoprotective properties (Pal *et al.,* 2001). It is also strongly alleged that turmeric can improve digestion and nutrient metabolism. It can also improve the function of digestive tracts (small intestine).

CONCLUSION

Turmeric supplementation non-significantly improved the measured growth and feed utilization parameters especially in the higher supplementation level (0.50%). The challenge infection using *P. fluorescens* revealed lower mortality percentage in the turmeric supplemented groups with highest level of protection in the 0.50% turmeric supplemented group. Further studies are needed to find out the effective use of turmeric with special reference to the timing, dosage, method of administration, and suggested mode of action in fish.

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