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Subfertility effects of turmeric (*Curcuma longa*) on reproductive performance of *Pseudotropheus acei*

Seval Bahadir Koca[a,*], Omer Ongun[b], Ozlem Ozmen[c], Nalan Ozgur Yigit[a]

[a] Isparta Applied Sciences University, Egirdir Fisheries Faculty, Isparta, Turkey
[b] Suleyman Demirel University, Graduate School of Natural and Applied Sciences, Aquaculture Department, Isparta, Turkey
[c] Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, Burdur, Turkey

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ABSTRACT

This study was conducted to determine the effect of turmeric powder dietary supplementation on the histology of visceral organs and reproductive performance of *Pseudotropheus acei*. The fish were fed dietary additions of 0%, 1%, 3%, 5%, and 7% turmeric powder. Each treatment was replicated three times. There were one male and four female fish with a mean weight of 6 g in each replicate. The fish were fed *ad libitum* for 90 days. There were no differences in fertility rate, fecundity rate, hatching rate, egg diameter, and larvae survival rate among groups ($P < 0.05$). Ovulation frequency and the percent of spawning broodstock were less in the 7% and 5% turmeric-supplemented group ($P < 0.05$), while there were no effects of treatment in the 1%, and 3% groups compared with the control group. Observations in histopathological examinations indicated there were normal tissue structures in the control, 1% and 3% turmeric-supplemented groups, while the addition of 5% and 7% turmeric induced a degeneration of and decrease in number of observable ovarian follicles. In addition, there was a normal liver structure in the control and the 1% and 3% turmeric-supplemented groups and slight to severe lipidosis in the 5% and 7% turmeric-supplemented groups. Also, the supplementation of larger amounts of turmeric induced enteritis and the slight to severe side effects on the relevant organs. These results indicate that supplementing of diets with turmeric powder in amounts of 5% and 7% suppressed ovarian follicle development, and extended periods between times of spawning which resulted in subfertility of broodstocks.

1. Introduction

Turmeric is a spice obtained from the rhizomes of *Curcuma longa*, a member of the Zingaberaceae ginger family (Chainani-Wu, 2003). Curcumin, an active component of turmeric, is a yellow pigment that has been isolated from the ground rhizome of the curcuma plant. Curcumin has anti-inflammatory, antioxidant, anticancerogenic and hypo-lipidemic properties (Tayyem et al., 2006). In addition, antifertility activities of turmeric (*C. longa*) have been reported (Trishna et al., 2010; Yadav and Jain, 2010; Sharma et al., 2013).

Sharma et al. (2013) reported that many of the plants had antifertility effects in females when consumed and were classified as containing estrous cycle disruptors, anti-estrogenic agents, anti-implantation agents, or abortifacient agents. Turmeric plants when consumed have estrous cycle disruptor effects (Priya et al., 2012; Sharma et al., 2013). Ghosh et al. (2011) reported that *C. longa*

* Corresponding author at: Suleyman Demirel University, Egirdir Fisheries Faculty, Cunur Campus, Isparta, Turkey.
E-mail address: sevalkoca@isparta.edu.tr (S. Bahadir Koca).

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induces suppression of behavioral estrous and ovulation in albino rats. Thakur et al. (2009) reported that *Carum carvi* and *C. longa* had significant antifertility activity in female albino rats. Yadav and Jain (2010) observed a complete inhibition of pregnancy in rats treated with *C. longa* [500 mg/kg body weight (BW)] extract. Furthermore, Trishna et al. (2010) reported that ethanolic and aqueous extracts of *C. longa* (200 mg/kg and 400 mg/kg BW) prevented pregnancy in rats.

In aquaculture, it may be desirable to prevent egg development so that there is not early sexual maturity associated with a smaller body size with the stunting of growth being due to the repartitioning of energy from body tissue to gonad development (Ekanem and Okoronkwo, 2003). Furthermore, uncontrolled reproduction (Kushwaha, 2013) can lead to a lesser amount of marketable product for aquaculture enterprises. The use of reproductive inhibitors, such as irradiation and chemosterilants, has disadvantages such as: being an expensive technology, the need for hatchery facilities with implementation requirement of skilled labor, and the expense and difficulty in procurement of hormones (Jegede, 2010). The use of reproductive inhibitory agents derived from plants for controlling reproduction in fish, therefore, may be economical and useful.

Various researchers have studied the use of plants for controlling reproduction of fish. Ekanem and Okoronkwo (2003) reported that when pawpaw seeds were fed to male Nile Tilapia permanent sterility was induced at a larger dose (9.8 g/kgBW/day), while there was a reversible anti-sterility effect with a smaller dose (4.9 g/kgBW/day). Kushwaha (2013) reported that indices for reproduction variables decreased with increasing dietary content of *Aloe vera* latex (0, 0.5, 1.0, 1.5, and 2.0 ml/kgBW diets) in Nile Tilapia. Jegede (2010) observed severe follicular atresia in the ovaries of Nile Tilapia fed a 4.0 g/kgBW diet addition of *Hibiscus rosa-sinensis* as a reproduction inhibitor. Obaroh et al. (2012) indicated that spawning was not observed in *Oreochromis niloticus* fed diets supplemented with 4.0 and 8 g/kgBW of *Azadirachta indica* Saponin.

The females of *Pseudotropheus acei* spawn on a flat rock, and after fertilization by the male, collect the eggs into her mouth. There is subsequent female incubation of the eggs for approximately 3 weeks before the fry are released from the mouth into the external environment (Bangerter, 2007). In the studies, with *Pseudotropheus acei*, there is an ease of administration of the test agent because of the fertilized eggs being present in the mouth of this species. The aim of the present study was to examine the antifertility effects of and organ reactions to *C. longa* in fish.

2. Materials and methods

2.1. Experimental diets

The experimental diets were isonitrogenous (37% crude protein) and isoenergetic (4400 kcal). Five diets were prepared by adding four different ratios of turmeric powder (T1: 1%, T3: 3%, T5: 5%, and T7: 7%) to a C: control diet with 0% turmeric powder. The composition of the diets is shown in Table 1. The feed ingredients were supplied by a local fish feed manufacturer. Turmeric powder was obtained from an established spice supplier in Isparta, Turkey. All ingredients were ground into small particles (0.5 mm) in a mill. Dietary ingredients were homogenized in a mixer. Micro-ingredients were first mixed and then slowly added to the blend of macro-ingredients to ensure a homogenous mixture. Water was added so as to have 30% moisture in the diet. Diets were passed through a mincer with a 1 mm sieve. The pellets were fan-dried and stored frozen at −20 °C until used.

2.2. Chemical analyses

The moisture, crude protein, crude fiber, and ash contents of the experimental diets were determined using the standard methods

Table 1
Formulation and proximate composition of experimental diets (%).

Ingredients	C	T 1	T3	T5	T7
Turmeric	0.00	1.00	3.00	5.00	7.00
Fish meal	35.00	35.00	35.00	35.00	35.00
Soybean	22	22	22	22	22
Corn starch	7.00	6.00	4.00	2.00	0.00
Wheat	26	26	26	26	26
Fish oil	7.00	7.00	7.00	7.00	7.00
Vitamin mix[1]	1.00	1.00	1.00	1.00	1.00
Mineral mix[2]	1.00	1.00	1.00	1.00	1.00
Pellet binder	1.00	1.00	1.00	1.00	1.00
Chemical analyses					
Crude protein (%)	36.91	36.99	37.15	37.31	37.47
Crude lipid (%)	10.24	10.30	10.41	10.53	10.65
Crude fiber (%)	1.75	1.80	1.89	1.98	2.07
Crude ash (%)	9.33	9.33	9.33	9.33	9.33
Energy (kcal)	4426.33	4423.71	4418.47	4413.23	4407.99

Vitamin premix[1]; per kg, 4000,000 IU vitamin A, 480,000 IU vitamin D3, 40,000 mg vitamin E, 2400 mg vitamin K3, 4000 mg vitamin B1, 6000 mg vitamin B2, 40,000 mg niacin, 10,000 mg calcium D-pantothenate, 4000 mg vitamin B6, 10 mg vitamin B12, 100 mg D-biotin, 1200 mg folic acid, 40,000 mg vitamin C and 60,000 mg inositol; Mineral premix[2]; per kg 23,750 mg Mn, 75,000 mg Zn, 5000 mg Zn, 2000 mg Co, 2750 mg I, 100 mg Se, 200,000 mg Mg.

of the Association of Official Analytical Chemists (AOAC, 2000). Moisture was analyzed by oven drying at 105 °C for 24 h. Crude protein was determined by the Kjeldahl method with the assumption of the factor (N x 6.25). Crude ash was determined using a muffle furnace at 550 °C for 24 h. Crude fiber was determined by sample digestion with sulfuric acid (H_2SO_4) and sodium hydroxide (NaOH). Lipids were extracted from samples using a chloroform-methanol mixture using the procedures previously described by Bligh and Dyer (1959).

2.3. Rearing conditions and design

Pseudotropheus acei broodstocks were obtained from a local commercial aquarium (Afyonkarahisar, Turkey). Broodstocks were then transferred to the aquaculture laboratory of the Egirdir Fisheries Faculty, Isparta Applied Sciences University, Isparta, Turkey. Broodstocks (mean weight 6 g) were randomly stocked so that there were three replicates for each aquarium (*n*; females = 4; male = 1). There were12 female and three male fish allocated for each treatment in total. Shelters were placed into the aquariums (70 × 30 x 40 cm) containing the broodstock, and the water was well-aerated and filtered.

The water temperature was maintained at a mean of 27 °C. The aquariums were climate controlled with use of aquarium thermostat heaters. The dissolved oxygen rate ranged from 5 to 7 mg/L. The experimental groups were manually fed, *ad libitum,* twice daily (8:30 and 20:30). The fish were fed experimental diets for 90 days. Control, T1, T3, T5 and T7 groups received 40.9 ± 1.7, 39.1 ± 1.6, 40.4 ± 1.3, 38.9 ± 1.4 and 39.9 ± 1.8 g of feed, respectively. The aquariums were cleaned daily, and the residual feed and feces were removed by siphoning.

2.4. Reproductive performance

The females were assessed for spawning status daily, and eggs were gently removed from the buccal cavity of females when ovulation had occurred. Photos of eggs were taken with a smartphone and the number of eggs was determined by marking the photo and visually counting egg numbers. Fertilized eggs were separated based on the differential coloring of unfertilized and fertilized eggs. The diameters of eggs were measured with a micrometer using a microscope. The larvae were monitored to determine the effects of turmeric on the survival ratio of larvae for 7 days.

2.5. Histopathological examination

At the end of the study for histopathological examination, four female fish from each replicate were euthanatized (ms222 1gr/lt), and tissue samples were collected during the necropsy. Because of the fish and organs were small, the whole body was transversally cut into six pieces from head to tail. After slicing all parts of the fish were fixed in 10% neutral formalin. After routine processing of the formalin-fixed samples using automatic tissue processing equipment (Leica ASP300S; Leica Microsystem, Nussloch, Germany), the samples were embedded in paraffin, and 5 µm serial sections from six different body sections were prepared using a Leica RM 2155 rotary microtome (Leica Microsystem, Nussloch, Germany). All sections were subsequently stained with hematoxylin and eosin (H&E), and whole slides were examined using a light microscope. In addition, there was assessment of all tissue samples using histochemical procedures with periodic acid-Schiff (PAS) staining. Histopathological changes were graded in a blinded manner. Oocyte diameters and the thickness of the zona radiata were measured in randomly selected regions of at least 10 oocytes in the same developmental stages. In the morphometric analysis of the small intestines, the length and width of the villi were measured in each fish at 40 x with an Olympus CX41 light microscope. Five different villi were measured in each fish for statistical analysis. Morphometric evaluation was performed using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan).

2.6. Statistical analysis

Spawning brood stock (%), larvae survival rate (%) and fertility rate (%) data were not normally distributed when assessed using the methods of Anderson-Darling test. These data, therefore, were analyzed by Kruskal-Wallis test and differences in mean ranks were determined using the Bonferroni-Dunn test. Data for egg diameter and fecundity rate (%) were normally distributed, and were analyzed using a One-Way ANOVA, and the Tukey test was used to determine the differences between the groups. Arcsin transformation was applied to data for hatching rate and a One-Way ANOVA variance analysis technique was used to compare the groups. The Tukey test was used to determine the differences between means of groups (Sheskin, 2000).

3. Results

3.1. Reproductive performance of P. acei

The data for reproduction performance of *P. acei* and survival rate of larvae are included in Table 2. The diets supplemented with turmeric powder, when compared to the control, had no effect on the fertility rate, fecundity rate, hatching rate, egg diameter, and larvae survival rate on the 7th day of treatment ($P < 0.05$). The percentage of the spawning broodstock in the T5 and T7 group, however, was different from the other groups ($P < 0.05$). The ovulation frequency decreased with the increasing dosages of turmeric.

Table 2
Reproduction performance of *P. acei*.

Reproductive Performance (mean ± SEM)	C	T1	T3	T5	T7
Fertility rate (%)	98.20 ± 0.94	97.98 ± 1.00	98.54 ± 0.97	96.67 ± 1.66	100 ± 0.00
	10.3	10.5	12.2	6.0	16.0
Fecundity rate (%)	9.32 ± 0.78	10.07 ± 0.50	11.09 ± 0.88	9.01 ± 1.00	9.79 ± 1.13
Hatching rate (%)	86.31 ± 3.91	77.99 ± 4.12	86.08 ± 4.56	87.89 ± 4.69	73.36 ± 4.93
Egg diameter (mm)	2.12 ± 0.05	2.14 ± 0.02	2.15 ± 0.08	2.02 ± 0.09	2.03 ± 0.04
Spawning brood stock (%)	219.40 ± 10.00[a]	205.56 ± 9.87[a]	222.22 ± 11.10[a]	100.00 ± 11.50[b]	61.11 ± 7.76[b]
Rank average	11.5a	9.3a	12.2a	5.0b	2.0b
Larvae survival rate in 7th day (%)	92.13 ± 3.10	91.44 ± 4.00	95.09 ± 2.78	91.91 ± 3.66	90.08 ± 3.46
Rank average	13.1	12.5	16.8	13.1	10.3

Fertility rate (%) = (No. of fertilized eggs/No. of total eggs)*100.
Hatching rate (%) = (No. of hatched eggs/No. of fertilized eggs)*100.
Fecundity rate (%) = No. of eggs/body weight of female (gr).
Larvae survival ratio (%) = Survival rate (%) = Final larvae number/initial larvae number)*100.

3.2. Histopathology

The histopathological examination of the hepatopancreas tissues indicated there were normal tissue structures in the control group, and there was a relatively normal appearance in the T1 and T3 groups. There was slight to severe lipidosis in the T5 and T7 groups, characterized by empty vacuoles as a result of an artifact (removal of fat) in slide processing from the cytoplasm of the cells. The severity of the lipid accumulation was not related to the dose of the turmeric and histologically both groups had a similar appearance (Fig. 1).

Histopathological examination of the ovaries indicated numerous immature or degenerative follicles in the T3, T5, and T7 groups while tissue structures in the control and T1 groups were normal. Degenerative changes in tissue structures increased with increasing dosage of turmeric with the most severe lesions being observed in the T7 group (Fig. 2).

Microscopic examination of ovarian follicles occurred at five different stages and diameters of the oocytes were calculated. In stage I (immature stage) oocytes were irregular in shape and were between 57 and 62 µm in diameter without a zona radiata. In stage II, oocytes generally had an ellipsoid shape with a granular cytoplasm and were 165 to 179 µm in diameter throughout the long axis. In stage III, the oocytes had large cortical vacuoles with wavy margins and were 395 to 422 µm in diameter. These oocytes had zona radiata with a 2 to 4 µm thickness at this stage. In stage IV, the oocytes were 758 to 825 µm in diameter, generally globular in shape, with a 7 to 9 µm thick zona radiata. At the stage V, (maturation stage) oocyst diameter ranged between 832 and 877 µm, without nucleus and granules and the thickness of the zona radiata was less than at earlier development stages ranging from 5 to 6 µm. There were markedly greater numbers of oocytes in the stage I developmental stage as compared with later stages of the T5 and T7 groups.

Morphometric analysis of the intestines indicated the length and width measurements of the villi were not affected by treatment. In the T5 and T7 groups, however, there was an inflammatory reaction in the propria mucosa (Fig. 3). The extent of the lymphocytic reaction was greater in the T5 and T7 groups and goblet cells were in greater numbers in these groups indicating chronic

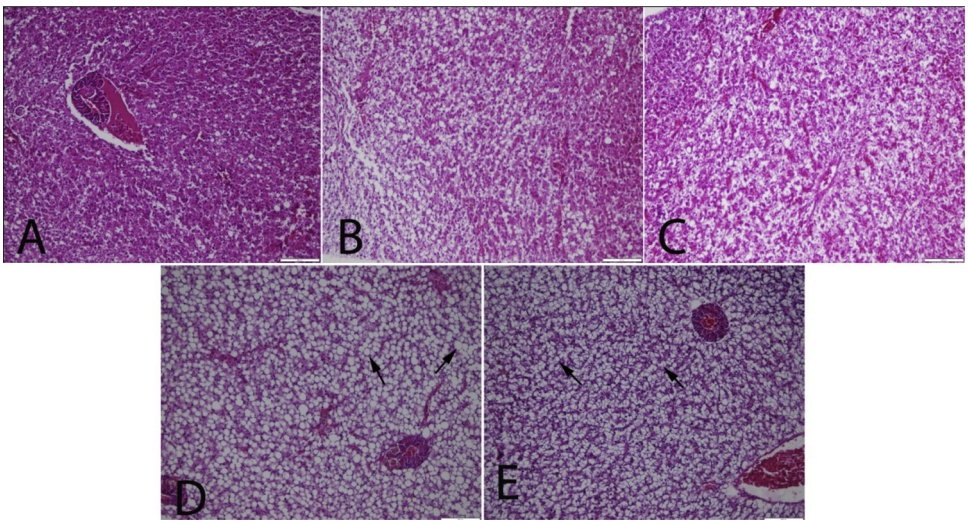

Fig. 1. Histopathological appearance of the hepatopancreases between the groups (A) Normal tissue structure in C group, very slight lipidosis in (B) T1 group and (C) T3 group; severe lipidosis (arrows) in (D) T5 group and (E) T7 group, HE; Bars = 100 µm.

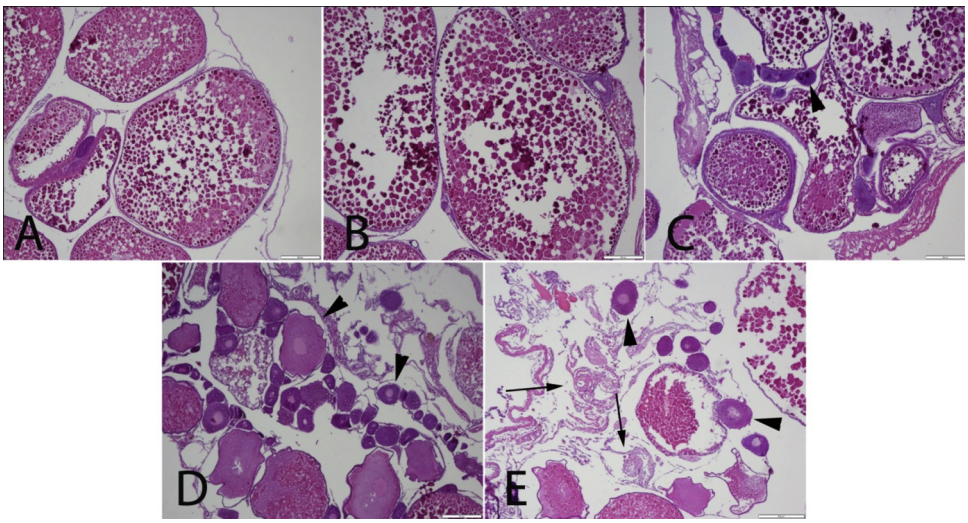

Fig. 2. Histopathological appearance of the ovaries in groups (A) Normal tissue architecture in C group; (B) relatively normal appearance in T1 group; (C) numerous immature follicles (arrow head) in T3 and (D) T5 groups; (E) degenerative follicles (arrows) in T7 group, HE; Bars = 100 µm.

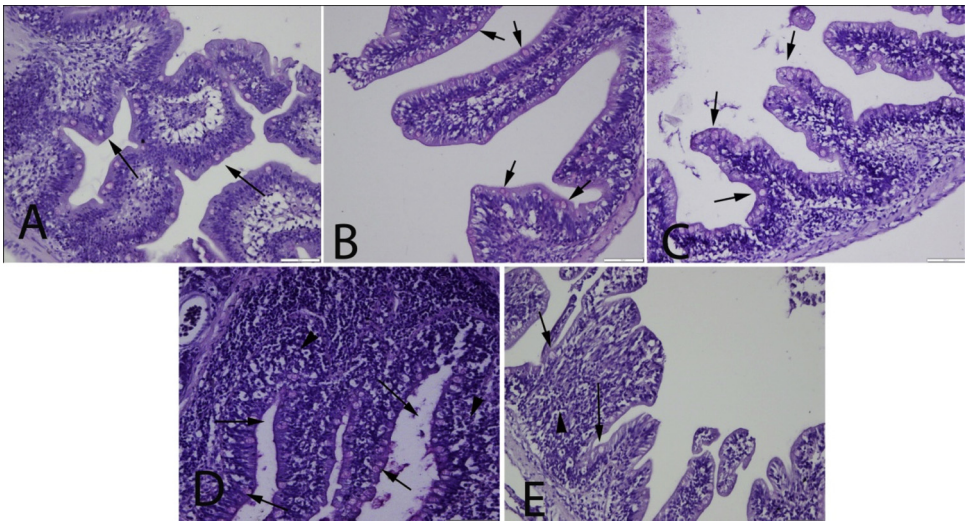

Fig. 3. Microscopic appearance of the gastrointestinal tissues (A) Normal tissue structure and PAS positive goblet cells (arrows) in C group; (B) relatively normal appearance in T1 group and (C) numerous goblet cells (arrows in T3 and (D) severe inflammatory cell infiltrations (arrow heads) in T5 groups and (E) in T7 group, PAS; Bars = 100 µm.

inflammation.

4. Discussion

To the best of our knowledge, this is the first study to evaluate the effects of turmeric on the reproduction performance of fish. The ovulation frequency was less as dosage of turmeric increased. Supplementation of 5% and 7% turmeric powder to diets suppressed the development of eggs. Maturation of oocytes, therefore, was delayed because of the reproductive cycle disruption. Specifically, mean egg numbers of an individual female did not change, however, the time elapsing between spawning periods increased as dosage of turmeric increased. There, therefore, were no significant differences in the larvae survival and fertility rates, fecundity rate, hatching rate, or egg diameter of broodstocks as a result of turmeric treatment. Trishna et al. (2010) reported that there were similar results when there was treatment with ethanolic and aqueous extract of *Curcuma aromatica* at two different doses, 200 and 400 mg/kg BW in the rats with there being an antifertility effect and prevention of pregnancy as a result of these treatments. Thakur et al. (2009) reported that *C. longa* had antifertility functions when there was treatment of female albino rats with this compound. Yadav and Jain (2010) observed that there was a complete inhibition of pregnancy in rats treated with *C. longa* extract (500 mg/kg BW). In contrast, Rahardja et al. (2015) reported that supplementation of turmeric powder to as much as 4% of the diet could lead to improvements

and maintenance of egg production of older laying hens and egg content of cholesterol was relatively less as compared with controls. Saraswati et al. (2013) reported that supplementing turmeric at 54 mg/quail/day improved values for egg quality variables. The addition of different ratios (0%, 2%, and 4%) of turmeric to diets of older chickens did not affect egg production or performance (Hassan, 2016).

The observations from histopathological examinations in the present study indicated that the lipidosis commonly observed in the hepatopancreases of the groups which had excessive curcumin added to their diets was characterized by empty vacuoles. The severity of the lipidosis was not related to the dosage of the turmeric in the T5 or T7 groups. The inflammatory reactions in the propria mucosa were observed in the T5 and T7 groups in the morphometric analysis of the intestines. Mahmoud et al. (2014) reported that the histopathology observations of the liver and intestines treated with turmeric indicated there was a mild degeneration with there being almost normal hepatic cords and intestinal villi, respectively, in *O. nilotucus* fed diets supplemented with 0.25% and 0.50% curcumin. Manju et al. (2012) confirmed the safety of curcumin as indicated by retention of a normal arrangement of the hepatocytes, hepatopancreases, and macrophage-melanocyte centers as assessed by histological analysis of fish (*Anabas testudineus*) fed diets supplemented with 0.5% and 1% curcumin. Yusuf et al. (2017) reported that adverse effects were observed on intestinal histomorphology of juvenile *O. niloticus* fed diets with 0.8% turmeric.

Generally, the feeding of turmeric or curcumin had a negative effect on the histopathology of tissues in previous studies. Similarly, in the present study, there were marked side effects of turmeric, especially in the T7 group, in the liver and gut because of its extensive metabolism in the intestine and liver. Excessive turmeric supplementation also affected the ovaries in this group. Ferreira et al. (2017), however, reported that there was no effect of turmeric on the frequency of the histopathological changes in the organ index and livers of juvenile *Astyanax aff. bimaculatus* fed diets supplemented with large dosages of turmeric powder (2%, 4%, 6%, 8%, and 10%).

The present study is the first report where there was histopathological assessment where there was evaluation of the effects of turmeric on fish ovaries. The feeding of large dosages of turmeric increased the immature or degenerative follicles in the ovaries. The ovulation frequency and spawning percentage of broodstock, therefore, was less when there was treatment with the larger doses. There were severe pathological effects in the ovaries related to turmeric supplementation. Future studies should focus on the possibility of reversible effects on eggs and organ damage when fish are fed with compounds that affect ovarian tissue functions. The oocyst in fish ovaries can be classified as stages I to V according to maturation status (Shabanipour and Hossayni, 2010). In the present study, the fertility and fecundity rates were similar but the total number of the larva was less as a result of treatments. Because there is only ovulation of maturate oocysts there were numerous ova that did not mature because of the reproductive cycle disruption with turmeric treatment in the present study similar to what has been previously reported (Priya et al., 2012; Sharma et al., 2013). These results indicate that supplementing diets with turmeric powder at 5% and 7% dosages resulted in a suppression of egg development and led to an extended time between spawning periods and the ultimate outcome was subfertility of broodstocks.

Conflict of interest

None.

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