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Assessment Study on the use of Pawpaw; *Carica papaya* Seeds to Control *Oreochromis niloticus* Breeding

Hossam H. Abbas and Wafaa T. Abbas
Department of Hydrobiology, Veterinary Research Division, National Research Centre, Egypt

Abstract: This study was carried out to assess the ability of using pawpaw (*Carica papaya*) seeds as a natural reproduction inhibitor for tilapia fish (*Oreochromis niloticus*) culture to control its breeding. Biochemical, physiological and histopathological effects of pawpaw seeds on male tilapia fish were also determined. Mature male tilapia were stocked for 4 weeks and treated with low dose (3 g/kg/day) and high dose (6 g/kg/day) of ground dried pawpaw seeds mixed with their feed. The obtained results showed that the pawpaw seeds induced permanent sterility in the high dose treated fish while the low dose treatment showed reversible results. The results also demonstrated that fish treated with high dose of pawpaw exhibited higher biochemical and physiological effects as: low meat quality, a progressive fall in erythrocyte (RBCs) count, hemoglobin (Hb) content and haematocrit values. Also the high dose revealed a significant increase in the leukocytes (WBCs) count, serum glucose, total protein, aspartate aminotransferase (AST), alamine aminotransferase (ALT), creatinine and uric acid levels. Moreover, serum total lipids revealed a significant decrease compared to control group. On the other hand, the low dose treatment revealed lower biochemical and physiological changes. Histological sections of testis showed disintegration of sperm cells and focal necrosis of seminefrous tubules in the high dose treated fish, hepato-pancreas and posterior kidney tissues also showed severe changes in high dose treated fish. Milder degenerative changes in some necrotic foci and slight changes in hepato-pancreas and posterior kidney were observed in the low dose treated fish. The study concluded that pawpaw seeds which are cheap and easy to obtain, can be incorporated into fish feeds with adjusted amount and be used to control breeding of tilapia fish in growing ponds instead of unfavorable and expensive hormonal use.

Key words: Aquaculture, pawpaw seeds (*Carica papaya*), nile tilapia (*Oreochromis niloticus*), breeding

INTRODUCTION

Tilapias are one of the most important fish species for freshwater aquaculture and represent a major protein source in many developing countries. Although endemic to Africa, their distribution has been widened by artificial introductions, mainly since the 1950's to include much of the tropics and subtropics (Pullin, 1997). Tilapia species constitute a major and important item in the Egyptian fish farming. Tilapias as a group displays many favorable attributes as cultured species. These include general hardness, resistance to diseases, high yield potential and ability to grow on a wide range of natural and cheap artificial foods. It also can withstand low oxygen concentrations, overcrowding, tolerate difficult ecological conditions and a wide range of salinities and still produce a highly acceptable flesh (El-Sayed, 2006).

Despite the many advantages of tilapia, it has often been described as a pest in Egyptian fisheries because of its precocious maturation and uncontrolled spawning which often result in overcrowding and stunting of the fish (Balarin and Hatton, 1979). So, the major set-backs in the intensive culture of tilapia species are their prolific fecundity. This leads to overpopulation of ponds, high competition and hence production of tiny fish.

Several methods for controlling Tilapia reproduction have been attempted such as combined stocking with piscivorous fishes, irradiation, monosex culture, cage culture, gynogensis and sex reversal (Beardmore, 1996). Of all the methods researched into, hormone induction of monosex populations seems to be the most promising and acceptable technique (Pandian and Varadaraj, 2005).

Hormonal control of fish sex is accomplished by administering a specific hormone to fry before sexual differentiation occurs. Gonadal differentiation of tilapia appears to occur between 8-25 days post-hatch (Nakamura and Takahashi, 1973). Since tilapia males grew faster than females, the culture of monosex male production is preferable and practiced widely in Egypt.

Using of hormones in any food products or additives is not desired from any consumers especially Egyptians. Tilapia fish has a bad reputation because of using

Corresponding Author: Wafaa T. Abbas, National Research Center, Veterinary Research Division, Department of Hydrobiology, Post Box: 12622, El-Tahrir Street, Dokki, Giza, Egypt Tel: +20 2 25272439, +2033370931

testosterone hormone in sex reversal, so scientists try deeply to find out some natural products that could do this job and make tilapia fish acceptable in the fish market. As the search for better solution to this problem continues, medical plants offer some possibilities. Medical plants have successfully been used to induce sterility in some laboratory animals (Gary and Garg, 1971; Das, 1980). Pawpaw (*C. papaya*) is a common man's fruit; available throughout the year. It is referred to as the medicine tree. Pawpaw seeds contain many active ingredients such as caricacin, an enzyme carpasemine, a plant growth inhibitor and oleanolic glycoside, the last of which had been found to cause sterility in male rats (Das, 1980; Kobayashi *et al*., 2008). Pawpaw (*C. papaya*) seeds contain antifertility properties, it can control the reproduction of male albino rats when administered orally (Udoh and Kehinde, 1999). Recently, some trials in Nigeria studied the effect of pawpaw seeds on fish and demonstrated its role to control the prolific breeding of Nile tilapia (Ekanemm and Okoronkwo, 2003; Ayotunde and Ofem, 2008). This method of control could be easier to adopt by poor fish farmers since pawpaw seeds are available all year around in the tropics and subtropical countries.

Biochemical and physiological biomarkers as well as some histological examinations are frequently used for detecting or diagnosing the effects in fish exposed to different substances (De La Torre *et al*., 1999). Such effects might lead to irreversible and detrimental disturbances of integrated functions such as behavior, growth, reproduction, meat quality and survival (El-Naga *et al*., 2005).

The objectives of this study were to assess using of pawpaw (*Carica papaya*) seeds in Egyptian *Oreochromis niloticus* aquaculture to control its breeding, As well as to determine its effect on some biochemical, physiological and histo-pathological aspects of tilapia fish.

MATERIALS AND METHODS

Fish aquaria: The study was in June, 2010. *Oreochromis niloticus* (*O. niloticus*) were obtained from Abbassa Fish Farm, Agricultural Research Centre, Abo-Hammad, Sharkia governorate, 85 km north of Cairo and acclimated in glass aquaria for two weeks during which the fish were fed on a pelleted feed (25% protein) at 26°C. The study was done in nine aquaria (three replicate for each treatment) and water quality criteria of the experiment is represented in Table 1. After acclimation, five males and five females of *O. niloticus* (approximately three months age) with a mean weight of 38±5 g and mean length 10.3±1.2 cm were stocked in each of the aquaria of 100×50×40 cm dimension.

Table 1: Physicochemical characteristics of water used in the experiment

Characteristics	Mean	Characteristics	Mean
pH	6.9	Bicarbonate (HCO_3^-)	129.4 mg L^{-1}
Temperature	26°C	Carbonate (CO_3^-)	0.00 mg L^{-1}
Dissolved Oxygen	5.8 mg L^{-1}	Sulfate (SO_4^-)	94.7 mg L^{-1}
Alkalinity ($CaCO_3$)	138.3 mg L^{-1}	Chloride (Cl^{-1})	19.9 mg L^{-1}
Total Hardness ($CaCO_3$)	132.5 mg L^{-1}	Calcium (Ca^{++})	34.9 mg L^{-1}
Ammonia (NH_3)	0.2 mg L^{-1}	Magnesium (Mg^{++})	7.6 mg L^{-1}
Ammonium (NH_4^+)	0.4 mg L^{-1}	Potassium (K^+)	5.1 mg L^{-1}
Nitrite (NO_2^-)	0.0 mg L^{-1}	Sodium (Na^+)	38.4 mg L^{-1}
Nitrate (NO_3^-)	1.2 mg L^{-1}	Electrical Conductivity	0.4 mmohs cm^{-1}
Total soluble salts	296 mg L^{-1}		

Preparation and introducing of pawpaw seeds: Sixty grams of ground pawpaw seeds which obtained from a private nursery orchard were added to 1 kg of the control feed ration to prepare the low dose treatment while 120 g of ground pawpaw seed was added to 1 kg of the control feed to get the high dose treatment. The dough of grounded pawpaw seeds was formed by adding water, mixed with the pelleted ration and then dried in the sun and stored.

Feeding of the fish on low and high dose pawpaw seed lasted for 30 days. The fish were fed at 5% of their body weight daily, in two installments daily. Fish in the first three aquaria were fed with the control feed without any additives (Group I); those in the second set of three aquaria were fed with the low dose treatment feed (Group II) while fish in the last set of aquaria were fed with the high dose treatment feed (Group III). At that feeding rate, the pawpaw seed dosage for the low dose treatment was 3 g kg^{-1} of fish/day while the high dose treatment was 6 g kg^{-1} of fish/day. The 96-h LC_{50} of pawpaw seed powder to adult tilapia is 4.2 mg L^{-1} (Ayotunde and Ofem, 2008).

Spawning of fish in all aquaria was observed daily as the experiment proceeded. At the end of the 30-days treatment period, the fish were weighted, nine male fish from each treatment and the control were sacrificed and blood samples, muscle tissue and organs were removed for sectioning and histological examination. The remaining fish in all aquaria were fed with the control feed and observed for another 30 days. Spawning was observed in all aquaria during these second 30 days and then the fish were weighed again before the experiment was finally terminated.

Hematological examination: Blood samples were withdrawn from the *Arteria caudalis* using a syringe containing sodium citrate as an anticoagulant. Samples were examined for the following: Total number of erythrocytes (RBCs) and leukocytes (WBCs) were counted using improved Neubauer Haemocytometer. Hemoglobin content was estimated using cyanmethemoglobin method (Van Kampen and Zijlstra, 1961). Packed Cell Volume (PCV) was carried out in small capillary haematocrit tubes using microhaematocrit centrifuge at 3000 rpm for 15 minutes. Blood indices namely Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC) were also calculated (Gupta, 1977).

Biochemical analyses

Muscle quality: Muscle chemical composition was determined using the following parameters: Muscle total protein was determined using the semi-microkjeldahl method (Josyln, 1950). Muscle ash was determined by burning samples in a muffle furnace for 16 h at 550°C and muscle water and lipids content were also determined (Sidwell *et al*., 1970).

Serum analyses: Blood samples were centrifuged at 3000 rpm to get serum for the following analyses: The level of serum glucose was measured using Boehringer Mannheim kits (Trinder, 1996). Total protein content was determined by Biuret test (King and Wootton, 1959). Total lipids level was determined colorimetrically by sulphova-nillin reaction. Serum aspartate aminotransferase (AST, E.N. 2.6.1.1) and alanine aminotransferase (ALT, E.N. 2.6.1.2) activities were determined colorimetrically using transaminases kits (Reitman and Frankel, 1957). Creatinine and uric acid were also measured (Henry *et al*., 1974). All the physiological parameters were measured by the S110 Auto-Zero Spectrophotometer WPA Linton, Camberidge UK.

Histopathological studies: Specimens from testis, livers and kidneys were fixed for 24 h in the formalin-saline solution (1:1 of 10% formalin and 0.9% sodium chloride). Histological sections of 5μ thickness were prepared following standard procedures (Luna, 1992).

Statistical analyses: The results were statistically analyzed using the T-test, analyses of variance (F-test) and Duncan's multiple range tests to determine difference in means (Duncan, 1955).

RESULTS

Spawning neither occurred in low nor high dose treated aquaria during the first 30 days treatment period whereas spawning was observed in the control group two weeks into the experiment. During the second 30 days, there was still no spawning in the aquaria that earlier receive the high dose treatment of the pawpaw seed. On the other hand, the previously low dose treated group showed spawning at the same period. Spawning recurred in the fifth week into the fish in the control aquaria. In the control experiment 68 to 76 fry were counted during the second spawning while 52 to 60 fry were counted in the previously low dose treatment aquaria.

Table 2: Muscle chemical composition of the Nile tilapia; *Oreochromis niloticus* treated with pawpaw seeds for 30 days

Treatments	Water contents (%)	Total protein (% of wet weight)	Total lipids (% of wet weight)	Ash (%)
Control	80.6±1.2[a]	15.9±0.31[a]	3.65±0.24[a]	1.95±0.12[a]
Low dose	81.8±0.53[a]	14.60±0.45[b]	4.55±0.19[b]	1.91±0.13[a]
High dose	83.9±0.31[b]	13.15±0.71[c]	4.26±0.15[c]	1.89±0.15[a]
F-values	210**	161**	96**	0.88

Data are represented as means of nine samples±S.E, Means with the same letter for each parameter in the same column are not significantly different; otherwise they do (Duncan's multiple range test, Duncan, 1955), **Highly significant difference ($p<0.01$)

Fish that receive pawpaw seed treatment showed a decrease in weight during the 30-day treatment when compared to the control (44.2±4 g for the low dose treatment and 47.1±6 g for the control), this difference was statistically non-significant ($p<0.05$). Fish that receive high dose treatment gained some weight after the treatment had been discontinued (45.5±4 g); however, the weight difference was not significant ($p<0.05$).

The biochemical results demonstrated that fish treated with high dose of pawpaw exhibited the lowest meat quality (Table 2), a progressive fall in erythrocyte (RBCs) count, hemoglobin (Hb) content and haematocrit value. While the leukocytes (WBCs) count, serum glucose, total protein, aspartate aminotransferase (AST), alamine aminotransferase (ALT), creatinine and uric acid levels declared a significant increase (Table 3). Moreover, serum total lipids revealed a significant decrease compared to control group (Table 4).

Histological sections of testis showed that pawpaw seeds produced some pathological changes as disintegration of sperm cells and focal necrosis of seminefrous tubules in the high dose treated fish (Fig. 1a). On the other hand the low dose treatment showed milder form of degenerative changes with some necrotic foci (Fig. 1b). Moreover, feeding on the high dose of pawpaw seeds showed vacuolar degeneration and necrotic foci in hepatic tissue (Fig. 1d) and necrotic changes in the form of cloudy swelling associated with some pyknotic nuclei with hyperplasia of interstitial hemopoeitic tissue in posterior kidney (Fig. 1g). While the low dose treatment showed lower pathological effect on hepatopancreas and posterior kidney tissues compared to the control group (Fig. 1e, h).

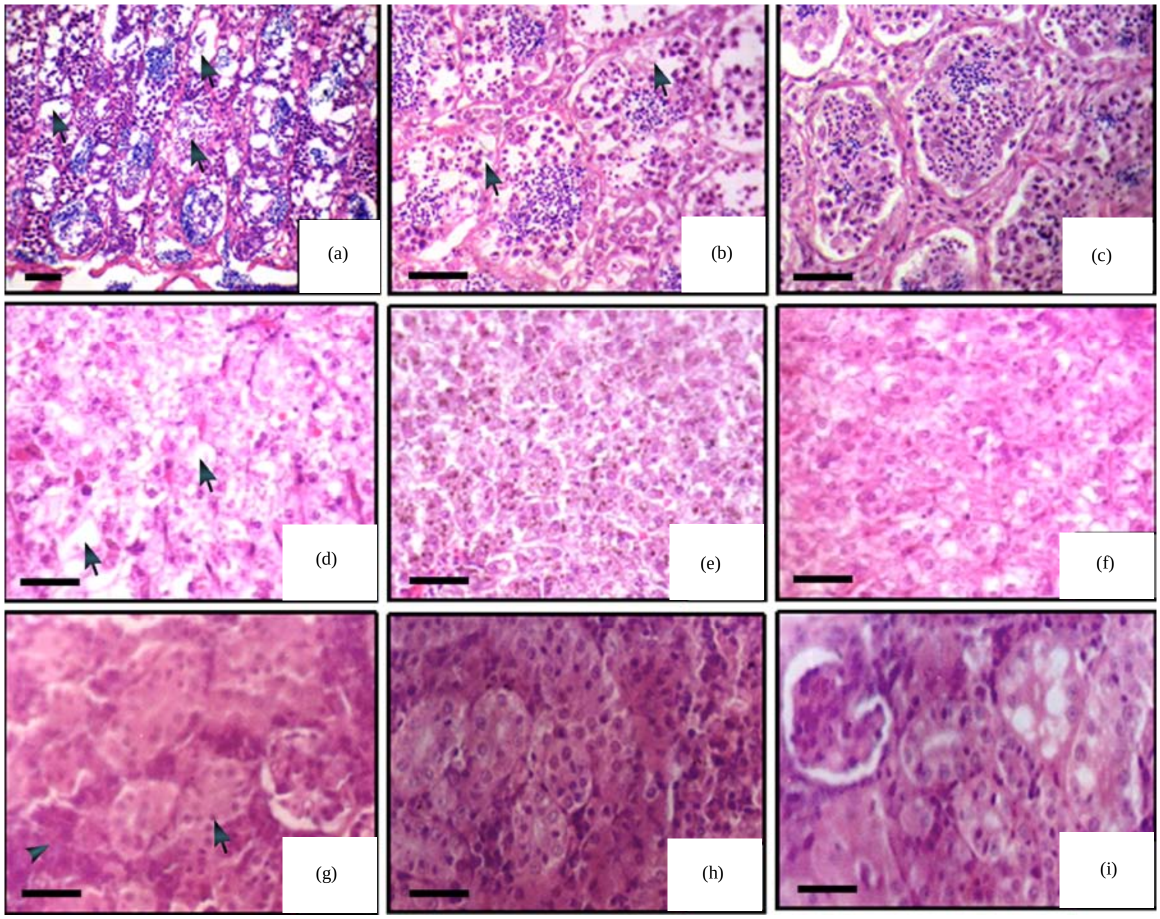

Fig. 1(a-i): Testis of *Oreochromis niloticus* showing some degenerative changes in seminefrous tubules in addition to the presence of focal necrotic areas (arrows) of the testicular tissue in high dose treatment (a), Milder form of degenerative changes and testis still showing the presence of focal necrotic areas (arrows) in low dose treatment (b), testis of control group showing normal structure (c), Hepatopancreas of *Oreochromis niloticus* showing some cellular vacuolation with the presence of necrotic foci (arrows) and increase of inter-sinusoidal spaces in high dose treatment (d), cloudy swelling of some hepatocytes in low dose treatment (e) and somewhat normal hepatic cells of control group (f), Posterior kidney of *Oreochromis niloticus* showing necrotic changes in the form of cloudy swelling associated with some pyknotic nuclei (arrow) with hyperplasia of interstitial hemopoeitic tissue (arrow head) in high dose treatment (g), Milder form of degenerative changes in renal tubular tissue in low dose treatment (h) and selected view of normal tissue in control group (i) (H and E, bar 50 μm).

Table 3: Blood parameters of the Nile tilapia; *Oreochromis niloticus* treated with pawpaw seeds for 30 days

Treatments	RBCs (X10^6 mm^{-3})	Hb (g 100 mL^{-1})	Ht (%)	MCV(μm^3 cell^{-1})	MCH(pg cell^{-1})	MCHC(g 100 mL^{-1})	WBCs(X10^3 mm^{-3})
Control	0.91±0.2^a	9.21±0.28^a	20.12±2.04^a	221.1±11.3^a	101.2±5.26^a	45.8±1.9^a	19.33±2.18^a
Low dose	0.86±0.05^a	8.32±0.51^b	19.01±1.9^b	221±7.8^a	96.7±2.3^a	43.8±2.2^a	21.43±1.11^a
High dose	0.73±0.04^b	7.58±0.47^b	17.83±2.22^c	244.2±9.5^b	103.8±3.7^a	42.5±1.5^b	24.86±1.78^b
F-values	411**	196**	211**	37**	41.6**	21**	124**

RBCs: Total number of erythrocytes, Hb: Hemoglobin content, Ht: haematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin and MCHC: Mean corpuscular hemoglobin concentration and WBCs: Total number of leukocytes, Data are represented as means of nine samples±S.E,
Means with the same letter for each parameter in the same column are not significantly different; otherwise they do (Duncan's multiple range test, Duncan, 1955), ** Highly significant difference ($p<0.01$)

Table 4: Serum constituents of the Nile tilapia; *Oreochromis niloticus* treated with pawpaw seeds for 30 days

Treatments	Glucose (mg 100 mL^{-1})	Total protein (mg 100 mL^{-1})	Total lipids (g L^{-1})	AST (U L^{-1})	ALT (U L^{-1})	Creatinine (mg 100 mL^{-1})	Uric acid (mg 100 mL^{-1})
Control	94.3±9.3^{a}	4.71±0.63^{a}	6.11±0.75^{a}	34.8±1.6^{a}	19.28±1.84^{a}	3.76±0.18^{a}	34.3±2.4^{a}
Low dose	100.8±4.2^{ab}	5.15±0.51^{b}	5.18±1.51^{b}	39.4±1.2^{b}	24.79±1.9^{b}	4.13±0.17^{b}	38.4±1.4^{b}
High dose	132.4±7.4^{c}	5.92±0.22^{c}	4.21±1.93^{c}	47.8±1.8^{c}	32.77±1.14^{c}	5.55±0.20^{c}	43.8±1.6^{c}
F-values	91**	5.11*	4.66**	133**	96**	109**	120**

AST: Aspartate aminotransferase and ALT: Alanine aminotransferase, Data are represented as means of nine samples±SE, Means with the same letter for each parameter in the same column are not significantly different; otherwise they do (Duncan's multiple range test, Duncan, 1955), *Significant difference (p<0.05), **Highly significant difference (p<0.01).

DISCUSSION

Re-spawning of the previously low dose treated fish after the treatment had been stopped revealed that pawpaw seed has a reversible effect on male *O. niloticus* sterility. This result was similar to that obtained by Ekanemm and Okoronkwo (2003) who studied the pawpaw seed as fertility control agent on male Nile tilapia. Such reversibility of low dose of pawpaw seed was also determined in case of male rat (Udoh and Kehinde, 1999). Moreover, low dose of pawpaw seed has the ability to control tilapia breeding and reduce the number of fry produced which was in similar to that results obtained in case of fish (Ekanemm and Okoronkwo, 2003) and rats (Gary and Garg, 1971; Udoh and Kehinde, 1999). The moderate changes observed in testis sections may discuss the reversible sterility effect of low dose of pawpaw seed on fish while the severe changes occurred to testis in high dose treated fish may explain the irreversible effect. Oleanolic glycoside is the ingredient responsible for sterility in rats (Das, 1980) while it needs more work to determine if that the same case in fish or not.

Decreased total muscle protein and total lipids of fish treated with pawpaw seeds might be attributed to the action of active ingredients of pawpaw seeds that may critically influence the growth rate and the quality of fish meat (Niragh and Simmone, 1984). The increase in muscle water content of treated fish especially those in high dose was explained by Weatherly and Gills (1987) who reported that depletion of body constituents (protein and lipids) results in tissue hydration of inverse dynamic relationships between protein as well as lipids and water content in the muscles. Similar results in some freshwater fishes were reported by Sakr (2001).

Hematological studies become promising tools for investigating physiological disturbances caused by any environmental changes and evaluation the fish health conditions. In the present study the treated fish showed decrease red blood cells count, hemoglobin content and haematocrit (Ht) values and an increase in the WBCs count. These findings therefore, coincided with those reported by Dabrowski *et al.* (1990), Li *et al.* (1998) and Sakr (2001). The latest author attributed the morphological changes in the erythrocytes and the decrease in Ht values to the reduction of hemopiosis due to the deficiency of some vitamins that play a role in iron metabolism and to intra-hepatic and intra-splenic hemorrhage. It has been found that, decrease in blood parameters (RBCs, Hb and Ht values) is accompanied by an increase in Mean Corpuscular Volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). This may be due to the hemolytic action that led to fluid loss to the tissues with subsequent decrease in plasma volume (Swift, 1981). Moreover, the increase in WBCs of treated fish was suggested to indicate alteration in defense mechanism against the action of active ingredients of pawpaw seeds lead to, external and internal hemorrhage with a reduction in rate of wound repair (Li *et al.*, 1998).

Biochemical analyses of serum constituents have proved to be useful in the diagnosis of metabolic disturbances and disease. Blood glucose appeared to be a sensitive and reliable indicator of environmental stress in fish. The reported hyperglycemia was attributed to glycogenolysis in liver due to the action of active ingredients of pawpaw seeds and/or to an increase in the plasma concentration of catecholamines and corticosteroids as a stress response of fish subjected to high dose of pawpaw seeds (Mazeaud *et al.*, 1977; Sakr, 2001). Moreover, the increase in serum total protein, AST and ALT activities in treated fish might be attributed to pathological conditions of liver and disturbances in liver due to the effect of active ingredients of pawpaw seeds (Ayotunde and Ofem, 2008; Kobayashi *et al.*, 2008). Also, increased serum total protein may be attributed to hemoconcentration or impaired water balance (Mazeaud *et al.*, 1977). On the other hand, the decrease in total serum lipids of treated fish was attributed to the increased secretion of catecholamines (Zaghloul, 2000) and corticosteroids (Mazeaud *et al.*, 1977) which enhanced metabolic rate and reduced metabolic reserves. Serum creatinine and uric acid could also be used as a rough index of the glomerular filtration rate. In the present investigation, treated fish showed high level of creatinine and uric acid. It might be attributed to the action of active ingredients of pawpaw seeds on the glomerular filtration rate (Sakr, 2001).

Histopathological examination of testis sections showed disintegration of sperm cells and focal necrosis of seminefrous tubules in the high dose treated fish while milder degenerative changes with some necrotic foci were observed in the low dose treated fish. This explains the permanent sterility occurred in high dose treated fish and the reversible effect in low dose treated fish. This was in agreement with results obtained by Ekanemm and Okoronkwo (2003) and Jegede and Fagbenro (2008) who revealed a severe effect of high dose of pawpaw seed on fish gonads. The severe histopathological changes were also recorded in liver and kidney tissues in case of high dose treated fish compared with low dose treated fish, this was in agreement with Ayotunde and Ofem (2008) who study the acute and chronic toxicity of pawpaw seeds on different organs of tilapia. This result was also in agreement with that carried out on rat by Kobayashi *et al*. (2008) and may reveal that the active ingredient of pawpaw seeds is strong chemicals that at a moderate dose can be effective as sterility-inducing agent but have a severe and damaging effect on fish organs at high dose (Das, 1980; Ekanemm and Okoronkwo, 2003). Moreover the higher biochemical parameters recorded in the high pawpaw concentration treatment may give an explanation to the more histo-pathological changes observed in that treatment compared with the lower one.

CONCLUSIONS

It could be concluded that using of pawpaw seeds which are cheap and easy to obtain, could be incorporated into fish feeds with adjusted amount and be used by farmers to control breeding of tilapia fish in growing ponds instead of unfavorable hormonal use but it should be supplied with known and accurate amount to avoid the adverse effects which could be occurred due to the strong active ingredients of pawpaw seeds. While, further studies need to isolate the pawpaw active ingredients and determine which one is responsible for the sterility and to avoid the toxicological effects of the other ingredients.

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