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Research Article

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Study of effect of papaya seed on reproductive performance in Nile Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The present study was conducted to examine the ability of papaya (*Carica papaya*) seeds in reducing the reproductive performance of Nile tilapia through gonadal sterilization. In the first phase, 9 days fry of Nile tilapia were reared in 50-L size aquaria and fed with normal feed (T_1) and papaya seed powder mixed feed @ 50 (T_2), 100 (T_3), and 150 (T_4) g/kg diet at the rate of 5 percent of body weight for 30 days. After 30 days of treatment, 50 fish from each group were reared in outdoor hapa for 6 months with normal feed, and gonadal status was observed. In the second phase, matured fish from both the control and papaya seed treated group (T_4@150 g/kg diet) were reared in two separate hapas for the next 3 months to observe their reproductive performance. Results showed that papaya seed feeding had no adverse effect on growth, survival, and water quality during the treatment period. The gonadosomatic index of both males and females was significantly lower in the papaya seed treated group (0.1±0.0 percent) than control (0.2±0.01 percent). The number of fish spawned per week was significantly lower in the papaya seed treated group (1.2±0.2) compared to the control group (3.6±0.3). The number of eggs per g female was significantly lower in the papaya seed treated group compared to the control group. The fertilization rate was significantly lower in the papaya seed treated group (93.6±1.1 percent) compared to the control group (98.2±1.3 percent) ($P<0.05$). This study demonstrated that feeding papaya seeds at the dose of 150 g/kg diet (15 percent) can reduce the reproductive performance of Nile tilapia.
Keywords: Papaya seed powder, a non-steroid aromatase inhibitor, masculinization.

INTRODUCTION

Nile tilapia (*Oreochromis niloticus*; Linnaeus, 1758) is a warm water fish with a greater aquaculture potential (Fitzsimmons, 2016). Some negative aspects of mixed-sex tilapia farming are a risk associated with their uncontrolled reproduction such as overcrowding, stunting, and possible escape from the fish farm, and genetic contamination with wild fish (Shrestha *et al*., 2011and Pandit *et al.*, 2015). Under aquaculture conditions, tilapia reaches sexual maturity early and starts reproducing with multiple annual spawning before they reach marketable size. Thus, there is an increasing demand for a reliable method to control reproduction in tilapia especially from aquaculture industries (Baroiller *et al.*, 2009). For profitable culture, various methods were conducted for the control of prolific breeding in tilapia and variation in the size of harvested fish. Various techniques to control unwanted reproduction in tilapia farming have been developed including stock manipulation (Phelps and Pompa, 2000), sterilization using chemicals (Ekanem and Okoronkow, 2003), polyploidy (Pradeep *et al.*, 2012), heat shock (Pandit *et al.*, 2015). However, each of these methods has its advantage and disadvantages. A reliable, non-chemical, consumer, and eco-friendly strategy for controlling reproduction in Nile tilapia seems necessary. Induction of sterility in Nile tilapia might be a good approach to control the reproduction and increase fish productivity (Pandit *et al.*, 2015). Similarly, Ekanem & Okoronkwo (2003) and Abdelhak *et al*. (2013) reported success in using papaya (*Carica papaya*) seed powder in inducing sterility in adult male Nile tilapia when administered through the feed. Papaya seeds contain 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). If we can apply this sterilization technique in smaller tilapia fry, it will be a great achievement to control reproduction in tilapia aquaculture. This method of reproduction control could be easier to adopt by poor fish farmers since papaya seeds are easily available all year round in the subtropical areas. The current investigation aimed to evaluate the optimum feeding dose of papaya seed in reducing the reproductive performance of Nile tilapia and also to assess the effect of papaya seed on water quality.

MATERIALS AND METHODS

This experiment was carried out at Agriculture and Forestry University (AFU), Rampur, Chitwan, Nepal from August 2016 to September 2017 which was conducted in two phases. In the first phase, 9 dAH (day after hatching) Nile tilapia (*Oreochromis niloticus*; GIFT Strain) fry was fed with papaya seeds for 30 days and grown to maturity stage for 6 months. In the second phase, the reproductive performances of papaya-treated fishes were studied throughout the breeding season for 3 months.

Brood fish management

About 50 well-matured brood fishes of 200 to 300 g were collected from the AFU aquaculture farm ponds. Brood fishes were maintained in 4 m x 4 m x 1.5 m nylon hapa in a cemented tank (5 m x 5 m x 1.5 m size) for breeding purposes. Both female and male fishes were stocked together in the hapa at a 2:1 ratio. Brood fishes were fed with commercial pellet feed @ 1 percent body weight ("Pusti" feed manufactured by Machhapuchchhre Feed Industry, Kapilvastu, Nepal, 28 percent crude protein). After being hatched when the yolk sac was absorbed and reached to first feeding stage 8-9 dAH were transferred to a 50-L aquarium and fed with different formulated feeds of subsequent treatments.

Experiment phase I

There were four treatments with three replications for each experiment. 9 dAH tilapia larvae were stocked in 50-L size aquaria (2 ft x 1 ft x 1.5 ft size) @ 100 fries/aquarium for papaya seed treatments. All aquaria were maintained in good aeration condition and 80 percent water was exchanged at each alternate day. The experiment was conducted in a completely randomized design with four treatments replicated thrice. The treatments were: T_1: Normal feed (rice bran and mustard oil cake 1: 1 ratio with 10 percent fish meal), T_2: Normal feed + 5 percent PSP (papaya seed powder), T_3: Normal feed + 10 percent PSP and T_4: Normal feed + 15 percent PSP.

Papaya seed (*Carica papaya*) local cultivar was collected and dried papaya seeds were then powdered with the grinder and sieved through a 60 µm mesh size sieve. Feeds containing 35 percent CP based on each treatment were formulated. The fishes were fed @ 5 percent of their body weight daily, in two installments at 1000 and 1500 hours. The feeding rate was adjusted based on the weekly sampling weight of fishes. Water quality parameters like dissolved oxygen (DO), pH, and temperature of the aquarium water were measured by DO meter and pH meter daily. Similarly, total ammonium nitrogen (TAN) and nitrate-nitrogen (NO_2-N) were analyzed on each alternate day just before and after water exchange.

Grow out or fish rearing phase was carried out in outdoor hapa maintained in cemented tanks. After 30 days of treatment in the aquarium, 50 fishes from each aquarium were stocked randomly into 12 nylon hapa (2 m x 1 m x 1.5 m) of 1 mm mesh size suspended in a 5 m x 5 m x 1.5 m cemented tank. Three cemented tanks were used for this purpose and each tank holding four hapas. Pellet feed ("Pusti") was fed for all treatments. The fish were fed at the rate of 2 percent of their body weight once a day. Fish were sampled monthly and the feeding rate was adjusted. Partial water exchange of three tanks was done fortnightly. Water quality parameters like DO, temperature, pH, and Secchi disc visibility were measured. All fishes in the hapa were reared for 6 months their maturation. After 6 months of culture, i.e., at the end of this phase, half of the fish from each hapa were dissected and gonadal observation was made morphologically and histologically.

Experiment phase II

Matured fishes (70-150 g) raised on control and papaya seed treated group were reared in two separate hapas (3 m x 3 m x 1.5 m) to observe their reproductive performance. In one hapa, 12 females and 18 males from the control group were stocked together. In another hapa, 12 females and 18 males from the treatment group (T_4) were stocked together. Low dose treatments groups (T2 and T3) were discarded for reproduction study because of the low proportion of abnormal gonad in these groups. Similar rearing conditions were maintained for both hapas. Broods were fed with commercial pellet feed ("Pusti", 4.0 mm size) at the rate of 2 percent body weight on daily basis. Water quality parameters such as DO, temperature, pH, and Secchi disc visibility were measured weekly. The breeding performance of brood fish was checked weekly by collecting eggs from the mouth and weighing and counting of collected eggs were done. The collected eggs were incubated in jars. During incubation, a regular supply of water was maintained in a way that eggs remained in continuous moving condition. Fry survival rate was calculated at 7 dAH. The parameters were observed are the number of fish spawned weekly, total number of eggs per unit body weight (BW) of fish, size (total length, TL) and weight of eggs, and hatching rate.

Data were statistically analyzed by using the one-way Analysis of Variance (ANOVA) and t-test. The statistical analysis was performed by using the computer software SPSS (Version 21.0). DMRT was used to evaluate the differences between means for treatments at the 5% level of significance. All means are presented with ± standard error.

RESULTS AND DISCUSSION

Experiment phase I

The average stock weight of juveniles in T_1, T_2, T_3, and T_4 were 0.26±0.11, 0.29±0.03, 0.24±0.03, and 0.16±0.07g, respectively (Table 1). The mean weight of fry at the end of the treatment period in T_1, T_2, T_3, and T_4 were 1.43±0.32, 1.33±0.06, 1.31±0.27, and 1.27±0.51g, respectively, without any significant difference among all treatments ($P<0.05$). Similarly, the mean survival rate of fry at the end of the treatment period in T_1, T_2, T_3, and T_4 were 63.3±8.9, 70.0±3.2, 73.0±10.6, and 73.0±13.2 percent, respectively, without any significant difference among treatments ($P<0.05$). The specific growth rate of fry at the end of the treatment period in T_1, T_2, T_3, and T_4 were 2.34±0.49, 1.93±0.17, 2.16±0.23, and 2.80±0.84 percent BW per day, respectively, without any significant difference among treatments (Table 1). The daily mean and range of water temperature, DO, pH, total ammonium nitrogen, and nitrate nitrogen in each treatment during the treatment period are given in Table 2. The temperature, DO and pH varied between 27.6 to 27.8 °C, 5.2 to 5.3 mg/L, and 7.4 to 7.5, respectively, in different treatments. There were no significant differences in temperature and DO concentration among all treatments. Similarly, the total ammonium nitrogen and nitrate nitrogen varied between 0.2 to 2.9 and 0.06 to 1.32 mg/L, before and after water exchange, respectively, in all papaya seed treated groups which do not significantly differ with control. There were no significant differences in total ammonium nitrogen and nitrate nitrogen among all treatments.

Table 1. Growth and survival of fry in different treatments during papaya seed treatment period.

Parameters	Treatments			
	T_1 (Control)	T_2 (5% PSP)	T_3 (10% PSP)	T_4 (15% PSP)
Total stock number	100±0.0	100±0.0	100±0.0	100±0.0
Total stock weight (g)	25.93±11.31	28.97±3.07	24.43±2.91	16.47±7.37
Average stock weight (g/fish)	0.26±0.11	0.29±0.03	0.24±0.03	0.16±0.07
Total final number	63.33±8.89	70.0±3.15	73.0±10.58	73.0±13.15
Total final weight (g)	86.19±9.46	93.35±5.37	89.9±4.52	86.89±10.05
Average final weight (g/fish)	1.43±0.32	1.33±0.06	1.31±0.27	1.27±0.51
Specific growth rate (% BW/day)	2.34±0.49	1.93±0.17	2.16±0.23	2.80±0.84
Survival rate (%)	63.33±8.89	70.0±3.15	73.0±10.58	73.0±13.15

Table 2. Mean and range of water quality parameters in different treatments during papaya seed treatment period

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Water temperature (°C)	27.6±0.3 (25.5-31.3)	27.8±0.3 (25.5-31.2)	27.8±0.3 (25.3-31.6)	27.8±0.3 (25.4-31.5)
Dissolved oxygen (mg/L)	5.2±0.2 (3.9-7.3)	5.3±0.2 (4.1-7.2)	5.2±0.2 (3.9-8.0)	5.2±0.2 (4.0-7.1)
pH	7.5 (6.8-7.8)	7.4 (6.8-7.7)	7.5 (6.8-7.9)	7.4 (6.8-7.7)
Total ammonium nitrogen before water exchange (mg/L)	0.72±0.10 (0.16-1.66)	0.65±0.07 (0.07-1.63)	0.77±0.10 (0.14-2.37)	0.75±0.03 (0.19-1.53)
Total ammonium nitrogen after water exchange (mg/L)	1.0±0.56 (0.17-2.9)	1.1±0.53 (0.20-2.92)	1.0±0.32 (0.20-2.92)	1.1±0.43 (0.26-2.41)
Nitrite nitrogen (mg/L) before water exchange	0.01±0.00 (0.01-0.02)	0.02±0.00 (0.01-0.03)	0.02±0.00 (0.01-0.04)	0.02±0.00 (0.01-0.03)
Nitrite nitrogen (mg/L) after water exchange	0.02±0.02 (0.01-0.06)	0.02±0.01 (0.01-0.06)	0.02±0.02 (0.01-0.02)	0.01±0.00 (0.01-0.01)

Table 3. Mean total feed used, total papaya seed used and FCR in different treatments during papaya seed treatment period.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Total feed consumed (g)	76.8±6.6	86.0±3.2	79.7±2.2	71.6±2.9
Net fish yield (g)	50.5±7.4^{a}	56.8±6.6^{a}	57.4±3.0^{a}	62.6±6.2^{a}
FCR	1.7±0.3^{a}	1.5±0.1^{a}	1.4±0.0^{a}	1.2±0.1^{b}
Amount of papaya seed used (g)	0.0±0.0	4.3±0.3^{a}	8.0±0.3^{b}	10.7±0.4^{c}

Mean values with a different superscript in the same row are significantly different (P<0.05).

Experiment phase II

The average stock weight of fry in T_1, T_2, T_3, and T_4 were 1.43±0.32, 1.33±0.06, 1.31±0.27 and 1.27±0.51g, respectively (Table 4). The average weight of fish at the end of rearing period in T_1, T_2, T_3 and T_4 were 38.3±3.6, 35.2±7.0, 37.5±2.6 and 40.9±5.6g, respectively, without any significant difference among treatments (P<0.05). The survival of fry at the end of hapa rearing phase in T_1, T_2, T_3 and T_4 were 80.7±5.2, 81.3±5.8, 80.0±3.1 and 76.0±9.1 percent, respectively, without any significant difference among all treatments. The average daily growth rate of fishes in T_1, T_2, T_3 and T_4 were 0.21±0.02, 0.20±0.04, 0.21±0.01 and 0.23±0.03g/fish/day, respectively, without any significant difference among the treatments (P<0.05) (Table 4).

Fortnightly mean and range of water temperature, DO, pH, and Secchi disk depth in each treatment during the experimental period are given in Table 5. The average temperature, DO, pH, and Secchi disc depth varied between 14.8 to 32.1 °C, 4.5 to 8.3 mg/L, 6.9 to 8.9 and 26.0 to 35.5 cm, respectively. There were no significant differences in temperature, DO and Secchi disc depth among the treatments (P<0.05).

Table 4. Growth and survival of papaya seed treated fish in different treatments during hapa rearing phas

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Total stock number	50±0	50±0	50±0	50±0
Total stock weight (g)	62.98±12.24	61.17±2.87	58.99±10.35	57.29±19.36
Average weight (g/fish)	1.43±0.32	1.33±0.06	1.31±0.27	1.27±0.51
Total final weight (kg)	1.53±0.05	1.30±0.18	1.49±0.05	1.51±0.07
Average final weight (g/fish)	38.3±3.6	35.2±7.0	37.5±2.6	40.9±5.6
Daily growth rate (g/fish/day)	0.21±0.02	0.20±0.04	0.21±0.01	0.23±0.03
Survival rate (%)	80.7±5.2	81.3±5.8	80.0±3.1	76.0±9.1

Table 5. Mean and range of water quality parameters in different treatments during the hapa rearing phase.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Water temperature (ºC)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-31.9)	21.0±0.0 (14.8-32.1)
Dissolved oxygen (mg/L)	6.5±0.1 (4.5-8.3)	6.5±0.1 (4.5-8.3)	6.5±0.1 (4.5-8.3)	6.6±0.1 (4.5-8.3)
pH	8.1 (6.9-8.9)	8.1 (6.9-8.9)	8.1 (6.9-8.9)	8.1 (6.8-8.9)
Secchi disc depth (cm)	31.2±2.9 (26.5-34.4)	30.9±2.8 (26.5-35.0)	31.2±2.9 (26.0-34.5)	31.0±2.8 (26.5-35.5)

The mean final weight of fishes, GSI, and suspected sterility (abnormal gonad morphology) percent of papaya seed treated fishes in different treatments at the end of the hapa rearing phase is provided in Table 6. The GSI of fish in T_1, T_2, T_3, and T_4 were 0.02±0.01, 0.01±0.00, 0.01±0.00, and 0.01±0.00 percent, respectively. The GSI of fish in T_1 was significantly higher compared to other treatments (P<0.05), whereas, there was no significant difference among T_2, T_3, and T_4. The suspected sterility rate of fish in T_1, T_2, T_3, and T_4 were 0.0±0.0, 10.5±1.2, 15.5±1.8and 20.0±2.1 percent, respectively. Among all treatments, the sterility was significantly highest in T_4, intermediate in T_3, and lowest in T_2(P<0.05).

The reproductive performance of normal (control) and papaya seed treated fish is shown in Table 7. The number of fishes spawned per week was significantly higher in the control group (3.6±0.3) than in the papaya seed treated group (1.2±0.2) (P<0.05). The mean egg number per ~~g~~ gram of females in the control and treatment groups were 2.1±0.1 and 1.9±0.2, respectively. Similarly, the mean egg weight in the control and treatment groups were 6.6±1.1 mg and 5.3±0.6 mg, respectively. There was no significant difference in egg number per ~~g~~ gram female and egg weight between the control and treatment groups. The fertilization rate was significantly higher in the control group (98.2±1.3 percent) than the papaya seed treated group (93.6±1.1 percent) (P<0.05). The incubation period of eggs in the control and treatment groups were 65.2±1.1 and 64.0±1.0 hours, respectively without any significant difference between the two groups (P<0.05). The hatching rate was significantly higher in the control group (81.5±2.6 percent) than the papaya seed treated group (73.2±2.1%) (P<0.05). The fry survival rate at 7dAH in the control and treatment groups were 92.4±3.7 and 90.5±2.4 percent, respectively without any significant difference between the two groups (P<0.05). Mean final weight and gonadosomatic index (GSI) of control and papaya seed treated fish at the end of the experiment are provided in Table 8. The GSI of female fish was significantly higher in the control group (3.9±0.5%) than the papaya seed treated group (2.7±0.3%) (P<0.05). Similarly, the GSI of male fish was significantly higher in the control group (2.1±0.2%) than the papaya seed treated group (1.5±0.1%) (P<0.05).

Table 6. Mean final weight, gonadosomatic index, and sterility rate of papaya seed treated fish in different treatments at the end of hapa rearing phase.

Parameters	Treatments			
	T_1 (Control)	T_2 (5%PSP)	T_3 (10%PSP)	T_4 (15%PSP)
Mean final weight of fish (g)	38.3±3.6	35.2±7.0	37.5±2.6	40.9±5.6
Mean length (cm)	11.4±1.7	11.1±3.2	11.3±1.4	11.9±2.6
Gonadosomatic index (GSI, %)	$0.2±0.01^a$	$0.1±0.00^b$	$0.1±0.00^b$	$0.1±0.00^b$
Suspected sterility rate (%)	$0.0±0.0^d$	$10.5±1.2^c$	$15.5±1.8^b$	$20.0±2.1^a$

Table 7. Reproductive performance of control and papaya seed treated fish

Parameters	Control	Treatment
Number of fish spawned per week	$3.6±0.3^a$	$1.2±0.2^b$
Egg number per gram female	$2.1±0.1^a$	$1.7±0.1^a$
Average egg weight (mg)	$6.6±1.1^a$	$5.3±0.6^a$
Fertility rate (%)	$98.2±1.3^a$	$93.6±1.1^b$
Incubation period (hr)	$65.2±1.1^a$	$64.0±1.0^a$
Hatching rate (%)	$81.5±2.6^a$	$73.2±2.1^b$
Fry survival at 7 dAH (%)	$92.4±3.7^a$	$90.5±2.4^a$

Mean values with a different superscript in the same row are significantly different (P<0.05).

Table 8. Mean gonad weight and gonadosomatic index of control and papaya seed treated fish at the end of the experiment.

Parameters	Control	Treatment
Female		
Mean weight (g)	113.3±17.0	75.7±12.6
Mean length (cm)	18.1±0.8	15.8±0.7
Gonad weight (g)	$4.3±0.8^a$	$2.1±0.6^b$
Gonadosomatic index (GSI, %)	$3.9±0.5^a$	$2.7±0.3^b$
Male		
Mean weight (g)	116.5±35.7	92.7±3.8
Mean length (cm)	17.7±1.1	17.3±0.3
Gonad weight (g)	$2.0±0.2^a$	$1.4±0.1^b$
Gonadosomatic index (GSI, %)	$2.1±0.2^a$	$1.5±0.1^b$

Mean values with different superscripts in the same row are significantly different (P<0.05).

The present study aimed to control reproduction in Nile tilapia by inducing gonadal sterilization in sexually undifferentiated fry feeding with papaya seed. Active feeding on papaya seed mixed diet and good growth of fishes in all treatments during treatment showed that papaya seed powder is well accepted by Nile tilapia fries. This study also shows that feeding papaya seed up to 15% of the total feed has no adverse effect on the growth and survival rate of Nile tilapia fry. Weight gained, feed conversion ratio, specific growth rate, and survival rate were optimal in both control and papaya seed treatment groups. The survival rate of fry during the treatment period in the present experiment (70-73 percent) is comparable with the result of Shrivastav (2016) and higher than the result of Ranjan (2015) feeding with common carp testis. Similarly, the specific growth rate of fry during the treatment period in the present experiment (1.9-2.8 percent BW/day) is lower than those reported by Ranjan (2015) and Shrivastav (2016). Similar findings were also reported by Lakshman *et al.* (2014) and Thompson *et al.* (2003) in papaya seed treatment, where they reported that the administration of papaya seeds extract does not show much influence on the structural composition of the intestine and suggested that the papaya seeds extract acts as an antioxidant effect, as well as improve the lipid profile.

In the present study, values of all recorded parameters of water during papaya seed treatment were within the acceptable limits for growth and reproduction of Nile tilapia (Pillay and Kutty, 2005). The survival and growth of fish during the papaya seed treatment period were well. Fish looked active, healthy, and of good color. This shows that feeding papaya seed has no adverse effect on water quality parameters. Milstein & Svirsky (1996) also reported that feeding papaya seed powder has no adverse influence on water quality. In contrast to the present study, Ekanem and Okoronkwo (2003) reported some discoloration and damage of the liver of fish from the high dose treatment of papaya seed. In the present study, the total ammonium nitrogen ranged from 0.2 to 2.9 and 0.06 to 1.32 mg/L before and after water exchange, respectively in papaya seed treated groups which are not significantly different from the control group. Pompa and Masser, (1999) reported that massive mortality occurs when fish are suddenly transferred to the water with NH_3 concentration greater than 2 mg/L; however, mortality will be reduced to half or less when they are gradually acclimatized to a level as high as 3 mg/L for 3 or 4 days. In the present study, after water change total ammonium nitrogen level gradually increased from day 1 to day 3 in which fish gets better acclimatized. The temperature, DO and pH recorded during the hapa phase were also in an optimum range for tilapia growth and survival. This shows that feeding papaya seed powder has no adverse effect on water quality parameters during or after the treatment phase.

In this experiment, the gonad weights were recorded for both the control and papaya seed-treated groups. The result showed that the mean gonad weight of (T_1) control fish (4.3±0.8g) was significantly higher than the (T4) papaya seed treated fish (2.1±0.6g). Similarly, the mean GSI of (T1) control fish (3.9±0.5 percent) was significantly higher than the (T4) papaya seed treated fish (2.7±0.3 percent). While there is no significant difference between body weight in control (113.3±17.0g) and treatment groups (75.7±12.6g). Similarly, there is no significant difference in the case of body length of both controls (18.1±0.8cm) and treatment group (75.7±12.6cm). The GSI of both males and females was significantly lower in the papaya seed-treated group compared to the control. Significant differences obtained in males GSI was in contrast with previous studies conducted on using papaya seeds as a reproductive inhibitor for an experimental animal such as albino rats (Maniyannan *et al*., 2009) and rabbits (Lohiya *et al.*, 1999). They recorded insignificant differences in the testis weight after administration of papaya seeds with that of the negative control. On the other hand, a significant decrease occurred in GSI of females agreed with the finding of Jegede and Fagbenro (2008) and Temitope (2010) who reported a significant decrease in GSI of Nile tilapia females treated with other medicinal plants such as neem (*Azadirachta indica*) and Hibiscus (*Hibiscus rosa Sinensis*) leaf. Abdelhak *et al.* (2013) also reported that GSI of males was not significantly different among treatments fed with a high dose of papaya seed treated feeds which induced permanent sterility in Nile tilapia. Khalil et al. (2014) reported that the fish fed with 6 g papaya seed powder per kg diet for 30 days and 2 g papaya seed powder per kg diet for 60 days recorded the highest values of GSI of males and females, respectively. While the fish fed with 2 g papaya seed powder per kg diet for 30 days gave the lowest values of GSI of males and females among all treatments.

Very low spawning in terms of frequency and number of eggs occurred in the papaya seed treated group compared to the control group. Out of 12 females stocked, the number of fish spawned per week in the papaya seed treated group was 1.2 which is three times lower than the number of fish spawned in the control group (3.6 fishes/week). Similarly, the number of eggs spawned per gm female fishes was 1.7 in the papaya seed treated group can equal to control (2.1), indicating that the papaya seed treatment significantly reduces the fecundity of fish. This result is similar to that of Ekanem and Okoronkwo (2003) in Nile tilapia and Udoh and Kehinde (1999) in the rat. The fertilization and hatching rates were also significantly lower in the papaya seed treated group compared to the control group. However, papaya seed treatment has no adverse effect on egg weight, incubation period, and fry survival rate. In a similar anti-fertility study by Verma and Chinoy (2002), papaya seed extract was administered intramuscularly on male albino rats at 5 mg/kg/day for 7 days and this resulted in a severe decrease in the contractile response of epididymal tubules when compared with the control experiment. Akin-Obasola and Jegede (2016) also reported that milt volume and sperm count of Nile tilapia were higher in the control group and decreased with increasing concentration of *Gossypium herbaceum* in the diet.

In the present experiment, few fish were spawned in the papaya seed treated group. This might be due to the low dose or reversible effect of papaya seeds. After treatment fishes were kept under normal feeding conditions for about six months until maturation. Ekanem and Okoronkwo (2003) also reported the reversible effect of papaya seeds in the low dose treatments which was because the damage done to the testes was minimal and could be repaired within a few weeks. After the recovery month, sections of both ovaries and testis showed the possibility of reversible effects in low and medium doses treatments, while permanent sterility occurred in high-dose treatment. Ekanem and Okoronkwo (2003) reported the absence of spawning in aquaria received the high dose treatment of papaya seed after stopping the treatment for 30 days. Maniyannan *et al*. (2009) recorded restoration of proper spermatogenesis in the male albino rat after 120 days of recovery of papaya seeds treatment. Lohiya *et al*. (1999) recorded complete reverse in male rabbits administrated papaya seeds after withdrawal of the treatment. Pandit *et al.* (2015) reported that the germ cells in the gonads can proliferate and recover in the original position after rearing the fish in normal condition after short-duration high-temperature treatment. They explained that to achieve complete gonadal sterilization, 100 percent germ cells should have degenerated. Based on these findings, we can say that feed papaya seed up to 15 percent of the total diet cannot induce 100 percent sterility in Nile tilapia juveniles and the remaining germ cells recovered after transferring the fish in normal feeding conditions. Overall, the present results demonstrate that feeding papaya seed during the juvenile stage suppresses the reproduction performance of Nile tilapia either by partial sterilization or any other physiological action.

CONCLUSION

The present study comes to introduce papaya seeds, which are cheap and easily available, as a natural agent to control the reproduction of Nile tilapia and overcome the problem of early maturation, instead of expensive chemical hormones. Reproductive parameters such as gonadosomatic index, fecundity, spawning frequency, and hatching rate in Nile tilapia treated with papaya seeds at doses of 150 g/kg (15 percent) feed revealed sterility. This makes papaya seeds at the dose of 150 g/kg (15 percent) diet or higher dose are recommendable for use as sterility-inducing agents in sexually undifferentiated Nile tilapia fry.

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