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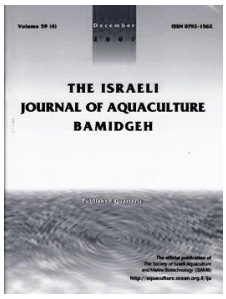

Effect of Pawpaw (*Carica Papaya*) Seed Meal on Sex Determination, Growth, and Survival, of *Oreochromis mossambicus* Fry

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Keywords: monosex; methyl testosterone; sex ratio; condition factor; histopathology; tilapia

Abstract

The production of monosex tilapia populations is a potential and effective solution for the precocious breeding and indiscriminate spawning that occurs in mixed sex culture systems. This study investigated the possibility of using *Carica papaya* seed meal (PSM) to skew the sex ratio of sexually undifferentiated *Oreochromis mossambicus* fry. A total of 2160 fry were used in the study, and fish were allocated to 12 treatment groups in triplicate, with 60 fish per replicate. The experimental diets fed included a basal diet (BD), 60mg methyl testosterone/kg BD, and 5 treatment diets containing 10, 15, 20, 25 and 30g PSM/kg of the BD, respectively for 30 and 120 days. PSM was able to skew the sex ratio in favor of males, with the proportion of males to females increasing with an increase in PSM content of the diet. The highest percentage phenotypic males (77.8%) induced by the PSM was obtained among the groups that received 20g and 30g PSM/kg BD. The growth and survival rates of the fish were not influenced by any of the treatment diets. Fragmentation of ovarian nuclei, hypertrophy of interstitial cells, and vacuolization of hepatocytes were observed in the gonads and hepatic tissues. The study demonstrated that an inclusion level of 20g PSM/kg BD was effective in converting females to phenotypic males.

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Introduction

Tilapia, endemic to Africa, is a species of choice in aquaculture systems, as they can tolerate a wide range of culture conditions. Their adaptability to different environment conditions allows this species to be exploited as a food fish for the increasing population of Sub-Saharan Africa. However, cost-efficient production is hampered by the precocious maturation and indiscriminate spawning of most tilapia species, which in turn results in overpopulation and consequent stunted growth and production losses (Toguyeni et al. 2002). The concept of mono-sex culture production of tilapia presents an opportunity to overcome the limitation of precocious breeding, and it is further supported by the fact that male tilapia grow faster than females (Guerrero et al. 1976, Phelps et al. 2000, Davis et al. 2010). The establishment of all-male tilapia populations prevents undesired indiscriminate wild spawning that stunt growth in fish ponds. Various methods are available for production of all-male tilapia populations and include manipulation of the immediate environmental temperature (Mires 1974, Baras et al. 2000), manual separation of sexes, genetic engineering, and hormonal induction. In gonochoristic fish like tilapia, phenotypic sex reversal can only be achieved in young fish with undifferentiated gonads. The most common commercially viable option in sex reversal in tilapia is the use of steroid hormones that are administered during the undifferentiated stage of gonadal development. Steroid hormones play a vital role in sex differentiation in tilapia (Shelton, 2006) and 17 α- methyl testosterone, a synthetic androgen when incorporated into the diet of sexually undifferentiated tilapia at an appropriate dosage and period, can convert genetic females to phenotypic males (Ridha and Lone 1990, Pandian and Sheela 1995). Despite the effectiveness of the synthetic hormone, concerns have been expressed concerning the health implication of the use of 17 α- methyl testosterone in food fish production on humans and on the aquatic environment. An alternative approach is to use natural substances that pose no environmental risk or health risk to humans, and which can manipulate the gender to obtain all-male tilapia populations.

Carica papaya (pawpaw) is a widely grown fruit tree in the tropics and subtropics. It belongs to the family *Caricaceae*, and according to studies, its fruit contains protein, fat, carbohydrates, vitamins A and C, carotene, minerals (calcium, iron and sodium), and fiber (Krishna et al. 2008). The different parts of this plant, including the seeds, are reported to possess antibacterial, antifungal, pesticidal, and anti-fertility properties (Mansura et al. 2009). Pawpaw seed has also been used as an anti-fertility agent in several animal models (Verma et al. 2006). An investigation on the potential of pawpaw seed powder when included as part of tilapia diets, produced monosex tilapia populations (Ampofo-Yeboah, 2013). In this study, we obtained a 65% masculinization rate in Mozambique tilapia (*Oreochromis mossambicus*).

The aim of this study was to determine the optimal inclusion level of PSM in the diet of undifferentiated Mozambique tilapia fry to obtain all-male populations. The effect of PSM on the growth, survival, and architectural integrity of the liver and the reproductive organs of the treated fish were also assessed.

Materials and Methods

Experimental location and facilities

The experiments were carried out in the water recirculation unit of the Aquaculture Research facilities based at the Welgevallen Experimental Farm of Stellenbosch University, South Africa. The recirculating aquaria system consisted of 72 glass aquaria tanks, each 57x53x40cm (LxWxH), and a capacity of 120.8L. Aeration and filtration components were incorporated into the design to ensure maintenance of optimal culture conditions throughout the trials.

Experimental animals and husbandry

A total of 2160 sexually undifferentiated *O. mossambicus* fry were used in this study. The fish were obtained from the Rivendell hatchery, Grahamstown South Africa. The age of the experimental animals varied between 1-2 weeks old, and the average weight and standard length of the fish was 0.036±0.014 g and 1.02±0.14 cm respectively. To avoid stocking a single progeny group in one treatment, fry were sorted

in bowls and randomly allocated to each treatment at a stocking density of 60 fish per replicate, and a total of 3 replicates per treatment. Prior to stocking the respective tanks, 10 fry were randomly taken from each replicate, and the individual weight and standard body length of each fish was recorded.

The physicochemical parameters (water temperature, dissolved oxygen, pH and conductivity) of the culture water were monitored daily. A digital YSI ProODO instrument with a fitted probe was used to monitor the dissolved oxygen. An YSI Ecosense conductivity meter (Model: EC300, YSI Inc., Yellow Springs, USA) was used to monitor the water temperature and conductivity. A Crison ICR12502 pH meter fitted with an ICR15053 electrode (HACH) was used to monitor the pH of the culture water. The recorded water quality parameters included; mean dissolved oxygen concentration of 4.98 ± 0.73 mg/L (range: 3.2 – 6.10), mean temperature 24.48 ± 1.46^{0}C (range: 22.2-26.70), Mean pH 6.42 ± 0.18 (range: 6.12-6.72), mean conductivity 210 ± 8.02 (range: 184.3-228.50).

Experimental diets and layout

Ripe pawpaw fruits were purchased from fruit vendors in Stellenbosch Western Cape, South Africa. The fresh seeds were removed from the fruit, and after rinsing in distilled water to remove the attached membranes of the fruit, the black seeds were spread open on black refuse bags and left indoors to dry. The dried seeds were then ground to obtain a powder using a laboratory grinder (Knifeter 1095, FOSS). The ground seeds were subsequently sieved through a laboratory sieve (SABS ISO3310, Model Minor, serial number: 371293, aperture: 500µm) to obtain a fine powder. The pawpaw seed powder (PSP) was packaged in zip lock bags, labelled and stored in a cool dry place until later use. The standard (basal) diet used throughout the study consisted of a commercial tilapia diet (Nutroscience (Pty) Ltd, Malmesbury, South Africa) of 25% crude protein level. Ingredient composition of the basal diet according to the production company include; protein – 400g/kg, lipids – 80g/kg, moisture – 120g/kg, fibre – 40g/kg, calcium – 30g/kg, and phosphorus – 7g/kg.

There were 12 experimental treatments based on the inclusion level of PSM and three replicates for each treatment. The experimental set up based on the inclusion levels and duration of the feeding period of the experimental diet was as follows;

i. Basal diet (BD) with no inclusion of PSM fed for 120 days was designated as P0M4 (control)
ii. BD + 60mg Methyl testosterone for 30 days
iii. Inclusion of 10g PSM/ kg BD fed for 30 days was designated as P10M1
iv. Inclusion of 15g PSM/ kg BD fed for 30 days was designated as P15M1
v. Inclusion of 20g PSM/ kg BD fed for 30 days was designated as P20M1
vi. Inclusion of 25g PSM/ kg BD fed for 30 days was designated as P25M1
vii. Inclusion of 30g PSM/ kg BD fed for 30 days was designated as P30M1
viii. Inclusion of 10g PSM/ kg BD fed for 120 days was designated as P10M4
ix. Inclusion of 15g PSM/ kg BD fed for 120 days was designated as P15M4
x. Inclusion of 20g PSM/ kg BD fed for 120 days was designated as P20M4
xi. Inclusion of 25g PSM/ kg BD fed for 120 days was designated as P25M4
xii. Inclusion of 30g PSM/ kg BD fed for 120 days was designated as P30M4

The experimental diets were mixed according to the different inclusion levels evaluated in the study, and all experimental diets were thoroughly mixed in a Macadams mixer (model: SM-401). To enable pelleting of the feed, 200mL lukewarm water was added per kg of feed during the mixing stage. The PSP-BD mixture was pelleted using an extruder (Trespade; model: 22 profess № 04/00090), and oven-dried in a CFW Envirowatch 5 oven (model: Ø560). The final pelleted diets were then packaged in zip lock bags and stored in airtight containers under cool dry conditions until later use. The quantity of feed prepared each time was such as to allow for maximum shelf life of two weeks. The pellets were ground to size suitable for the mouth size of the fish before feeding. The fish were fed *ad libitum* three times a day (9.00, 13.00 and 17.00h) with the experimental diets. The waste and uneaten food in the aquaria were carefully

removed daily by siphoning and the tanks refilled with fresh water. The culture tanks were checked daily for mortalities and the dead fish removed immediately. The experiment lasted 120 days when the fish might have reached sexual maturity. The groups that were fed the experimental diet for 30 days were maintained with the basal diet from the 31st day till end of the experimental period (120 days).

Sampling and Data recorded

The morphometric parameters of all fish in the different experimental groups were taken at the end of the experimental period (120 days). The standard lengths of the specimens were measured using a measuring board graduated in centimeters. Top loading balance (Electronic Balance, UWE, HGS-300, capacity: 300 x 0.01g, Serial # P9440) was used to measure body weight of the fish to the nearest gram. The mortality in different treatments was recorded as well as the number of females and males at the end of the experimental period. Condition factor, growth, and mortality rates were also determined.

The condition factor (K) of each fish was calculated using the formula

$K = 100W/ L^3$

Where L = standard length (cm) and W = Body weight (g)

Specific growth rate was calculated using the formula;

$$SGR = \frac{LnW_f - LnW_i \quad X100}{T \text{ (days)}}$$

Where W_f = final weight and W_i = initial weight

Survival rate = Final number of fish X100/ Initial number of fish

Identification of sexes

Females and males from each replicate were identified and all fish were weighed at the time of sexing. At the end of the experimental period when the fish size enabled visual sexing (Pandian and Varadaraj 1987), fish with one orifice on the genital papillae were recognised as males and those with two as females. The sexing was done with the aid of a hand lens, and each of the fish was also dissected to retrieve their gonads and confirm the sex by microscopy.

Histological evaluation of tissue samples

At the end of the experimental period, histology of the liver and gonads were undertaken to determine the effects of the phytochemicals on their architectural integrity. Gonad and liver tissue samples were collected from 4 fish (two males and two females) and 2 fish (1 male and 1 female), respectively from each replicate, and preserved in 10% buffered formalin until further processing. Processing of the slides for histological examination was performed according to standardized protocols at the Department of Physiology, University of Stellenbosch, South Africa. After the slides were mounted, all slides were graded according to a predetermined scale for gonad and liver integrity, respectively.

Statistical analysis

Sex ratio among different treatments was calculated. Significant deviation in sex ratio was analysed using Logistic regression and Chi- square test to ascertain the level of deviation from the expected 1:1 (M:F) ratio. Kolmogorov Smirnov's test was used to estimate normality of data and homogeneity of variance was assessed with Levene's test. Based on these tests, all data were found to be normally distributed. The data (mean± SD) of the morphometric parameters (standard length, and body weight) measured and the derived indices (condition factor, specific growth rate, and survival rate) were analysed using single factor analysis of variance (ANOVA). Variant means were separated using Bonferroni (Dunn) t test, and mean differences where $p<0.05$ was considered statistically significant. All statistical analysis was performed using SAS system ('local', X64_7PRO, Version 9.1).

Results

At the termination of the trial at day 120, the mean standard length of P25M1, P10M4, P30M4 and BDM4 were significantly shorter ($p<0.05$) than that of P20M4 while the mean weight of P25M1, P10M4 and P0M4 were also significantly lower ($p<0.05$) than those of P20M4. In addition, the Fulton's condition factor of P20M4, P25M4 were significantly higher ($p<0.05$) than that of P10M1, P10M4 and P15M4 (Table 1).

Table 1: Morphometric parameters (mean ± SD) of *O. mossambicus* fed diet containing pawpaw seed meal (PSM) and 17α-methyl testosterone during a 120 day culture period.

Treatment	*Standard length (cm)*	*Weight (g)*	*Condition factor*
P0M4 (control)	9.44 ^{b} ±0.86	34.52 ^{b} ±7.94	4.06 ^{ab} ±0.39
MTM1 (control)	10.02 ^{ab} ±0.62	39.57 ^{ab} ±5.66	3.94 ^{ab} ±0.45
Period 30 days			
P10M1	9.98 ^{ab} ±1.25	38.88 ^{ab} ±11.32	3.87 ^{a} ±0.69
P15M1	9.78 ^{ab} ±0.78	37.68 ^{ab} ±7.82	3.99 ^{ab} ±0.28
P20M1	9.81 ^{ab} ±1.02	38.68 ^{ab} ±9.42	4.03 ^{ab} ±0.35
P25M1	9.54 ^{b} ±0.82	34.58 ^{b} ±8.83	3.93 ^{ab} ±0.34
P30M1	9.81 ^{ab} ±0.81	38.13 ^{ab} ±8.03	3.99 ^{ab} ±0.28
Period 120 days			
P10M4	9.35 ^{b} ±0.76	35.11 ^{b} ±8.29	3.88 ^{a} ±0.3
P15M4	9.6 ^{ab} ±0.91	37.23 ^{ab} ±8.91	3.88 ^{a} ±0.31
P20M4	10.33 ^{a} ±0.69	43.2 ^{a} ±9.04	4.24 ^{b} ±0.51
P25M4	9.92 ^{ab} ±0.7	38.04 ^{ab} ±7.0	4.15 ^{b} ±0.3
P30M4	9.56 ^{b} ±0.79	36.08 ^{ab} ±8.77	4.07 ^{ab} ±0.36

$^{a, b}$ Columns with different superscripts differ significantly ($P < 0.05$)

Effect of Pawpaw seed meal on sex ratio

The identification of gender by means of the absence or presence of ovaries in their gonads could be performed with ease at the end of the 120-day trial period. The potential of PSM included as part of the basal tilapia diet, and of the 17 α -methyl testosterone to masculinize tilapia is presented in Table 2. In the negative control group (P0M4) which were fed the basal diet only, the male to female (M:F) sex ratio of 1.09:1 did not differ significantly ($p>0.05$) with the expected sex ratio of 1:1, however, there was a significantly higher ($p<0.05$) number of males to females among the positive control group (MTM1) which were fed the diets containing 60mg methyl testosterone per kg for 30 days. In all the treatments, the highest sex ratio of five males to a female (5: 1) was obtained in methyl testosterone treated group (MTM1). At an inclusion level of 10 g/kg of basal diet, PSM was able to skew the sex ratio in favor of males (60% males to 40% females) (Table 2). The proportion of males increased with an increasing dosage of pawpaw seed meal (PSM), with the maximum masculinization achieved at an inclusion level of 20 g/kg BD, resulting in 77.8% males produced. The M: F sex ratio obtained in the group fed 20g PSM/kg BD was significantly higher ($p<0.05$) than those of the negative control (no inclusion of PSM). Also the M: F sex ratio obtained in the groups P0M4, P10M1, P10M4, P15M4 were significantly lower ($p<0.05$) than the treatments MTM1, P15M1, P20M1, P25M1, P30M1, P20M4, P25M4, and P30M4. When the masculinization success of the diets containing 20 g PSM/kg BD and 30 g PSM/kg BD respectively, was compared, no significant differences ($p>0.05$) were observed in terms of the numbers of males produced. When the masculinization success was also compared

in terms of the duration of the feeding regimes of 30 days and 120 days, no significant differences (p>0.05) were observed in terms of the number of males produced (Table 2).

Table 2: The influence of pawpaw seed meal and 17α-methyl testosterone, included as part of a basal tilapia diet, to masculinize *O. mossambicus* fry after a 30 day and 120 day treatment period, respectively.

Treatment	*% Males*	*%Females*	*M:F*	x^2	*P-value*
P0M4 (control)	52.22	47.78	1.09: 1[a]	0.21	0.65
MTM1 (Control)	83.33	16.67	5: 1[b]	23.16	<0.0001
Period 30 days					
P10M1	60	40	1.5: 1[a]	2.69	0.10
P15M1	63.33	36.67	1.72: 1 [b]	4.37	0.037
P20M1	74.44	25.56	2.91: 1 [b]	13.03	0.000
P25M1	74.44	25.56	2.91: 1 [b]	13.03	0.000
P30M1	77.78	22.22	3.5: 1 [b]	16.54	<0.0001
Period 120 days					
P10M4	62.22	37.78	1.65: 1[a]	3.77	0.052
P15M4	62.22	37.78	1.65: 1 [a]	3.77	0.052
P20M4	77.78	22.22	3.5: 1 [b]	16.54	<0.0001
P25M4	74.44	25.56	2.91: 1 [b]	13.03	0.000
P30M4	75.56	24.44	3.09: 1 [b]	14.16	0.000

[a, b] Columns with different superscripts differ significantly (P < 0.05)

When the specific growth rate (SGR) of different treatment groups was compared, P20M4 had a significantly higher (p<0.05) SGR than P0M4 (control), P10M4 and P25M1 (Table 3). The inclusion of pawpaw seed meal in the diets did not affect the survival in most of the treatments. The exception was treatments P30M1 which had significantly lower (p<0.05) survival rate than those of treatments P0M4, MTM1, P10M1, P10M4, and P15M4. However, there was no distinct trend in the survival rates of different treatments that can be attributed to the effect of the experimental diet (Table 3).

Table 3: Survival and specific growth rates of *O. mossambicus* fed diet containing pawpaw seed meal (PSM) and 17α-methyl testosterone during a 120 day culture period.

Treatment	*Num. stocked*	*Num. Harvested*	*SurvivalRate (%)*	*SGR*
P0M4 (control)	180	162	90.00[a]	3.54[b]
MTM1 (control)	180	161	89.44[a]	3.68[ab]
Period 30 days				
P10M1	180	165	91.67[a]	3.66[ab]
P15M1	180	140	77.78[bc]	3.63[ab]
P20M1	180	136	75.56[bc]	3.66[ab]
P25M1	180	140	77.78[bc]	3.54[b]
P30M1	180	124	68.89[c]	3.64[ab]
Period 120 days				
P10M4	180	153	85.00[ab]	3.56[b]
P15M4	180	153	85.00[ab]	3.62[ab]
P20M4	180	139	77.22[bc]	3.77[a]
P25M4	180	138	76.67[bc]	3.64[ab]
P30M4	180	141	78.33[bc]	3.59[ab]

[a, b] Columns with different superscripts differ significantly (P < 0.05)

Effect of the PSM on the ovary and testes tissues

The pathology observed in the ovary includes fragmentation of the ovarian nuclei, hyperplasia of the follicular epithelium and atresia of the follicles (Fig.1). The effect on ovaries were observed in fish from treatment P30M1 fed the diet containing 30g of PSM/ kg of BD in which out of the six fish examined, two had the lesions, also in treatment P25M4 and P30M4 which were fed 25 and 30 g respectively of PSM/ kg of the basal diet.

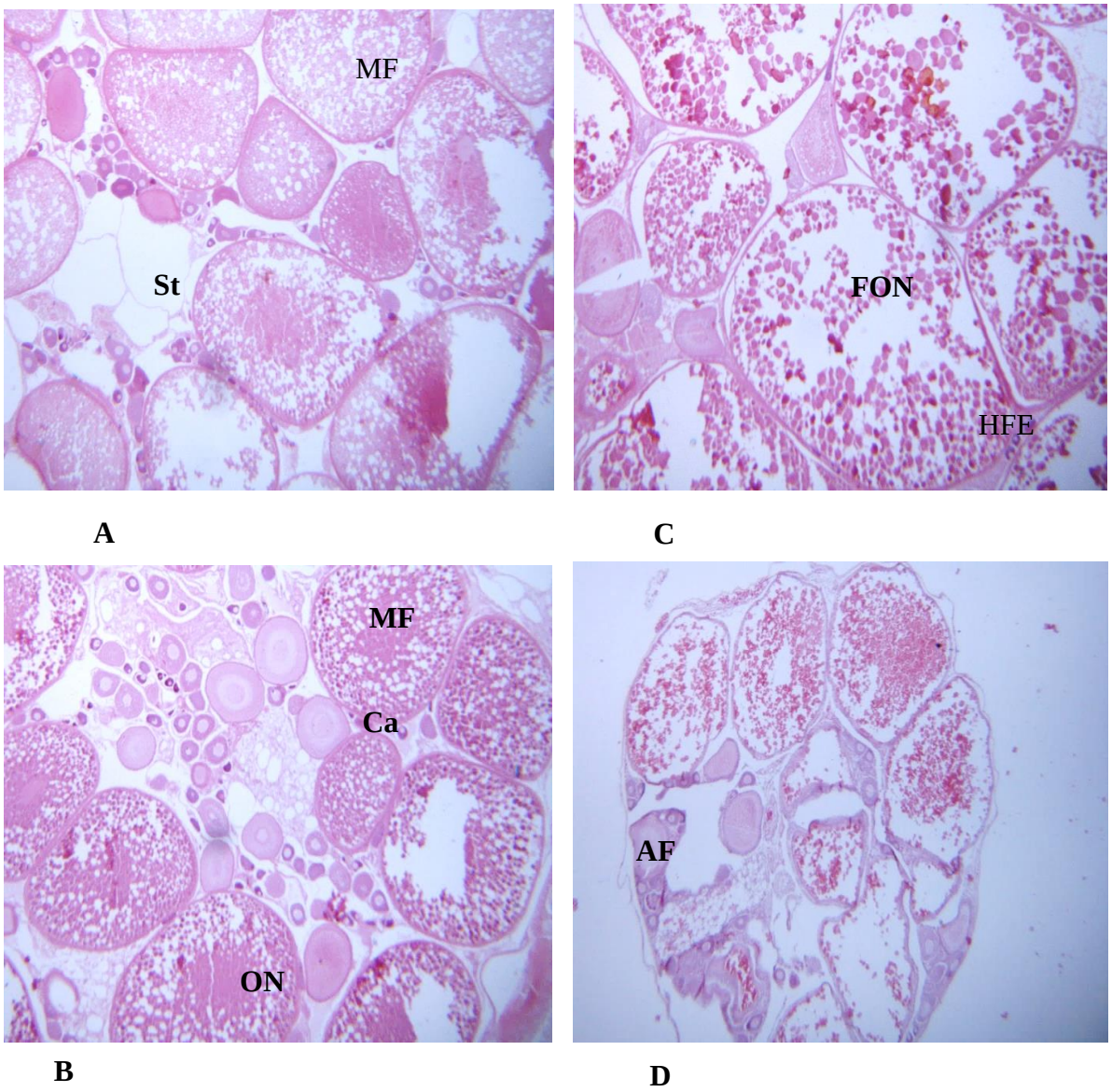

Figure 1 (A – D): *Oreochromis mossambicus* ovary showing the effect of *C. papaya* on the ovarian tissues (H&E staining, 400 X magnifications). A and B represent normal ovarian tissues of the group fed BD with no inclusion of PSM with follicles at various stages of development, while C and D represent ovarian tissues of the group fed 30g PSM/ kg of BD with certain pathological changes. MF – mature follicle; Ca – capsule; ON – ovarian nucleus; St – stroma; AF – atretic follicle; HFE – hyperplasia of follicular epithelium; FON – fragmentation of ovarian nucleus.

Two fish out of the six sampled had lesions in treatment P25M4 while three had them in treatment P30M4. The histopathological effect revealed in the testis included hypertrophy of the interstitial cells and mild vacuolation of the germ cells (Fig.2). One of the six fish examined in treatments P25M1, P30M1, and P25M4 had lesions while two of the six fish examined in treatment P30M4 had histopathological lesions in the testis.

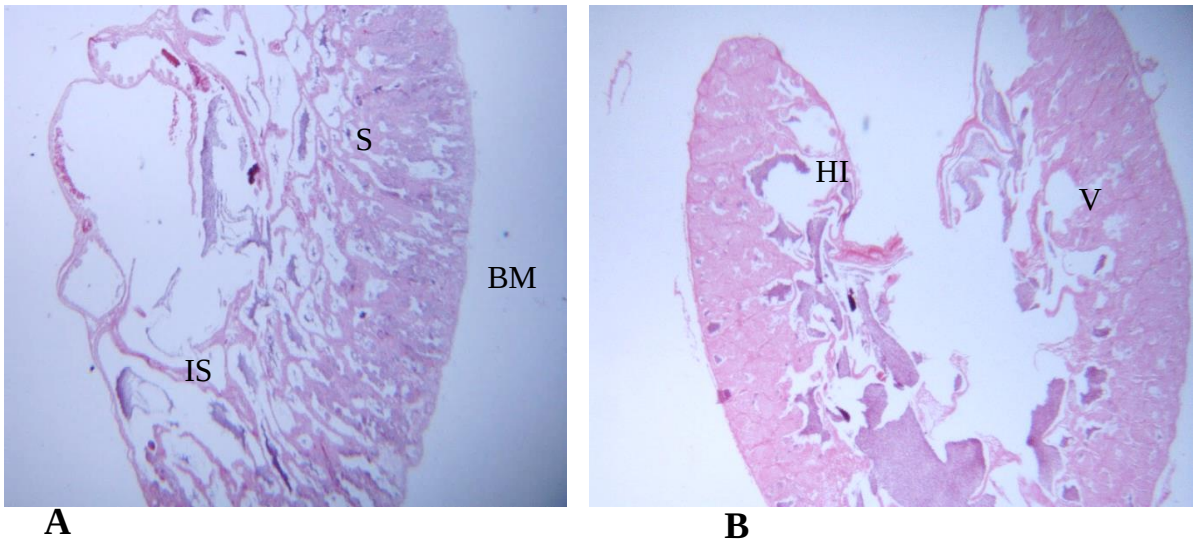

Figure 2 (A – B): An *Oreochromis mossambicus* testis showing the effect of *C. papaya* on the testicular tissues (H&E staining, 400 X magnifications). A represents normal testicular tissue of the group fed BD with no inclusion of PSM, while B represents testicular tissue from fish fed 30g PSM/ kg of BD. S – spermatocyte; IS – interlobular septa; BM – basement membrane; HI – hypertrophy of interstitial cells; AF – atresia of follicles; V - vacuolization of germ cells.

Effect of the PSM on the liver tissues

The pathological lesions observed in the ovaries included vacuolation of hepatocytes, dilation of sinusoid, and hepatic degeneration (Fig.3). One of the six fish examined in treatment P25M1 had lesions while two of the fish examined in treatments P30M1, P25M4, and P30M4 had pathological lesions in their liver tissues.

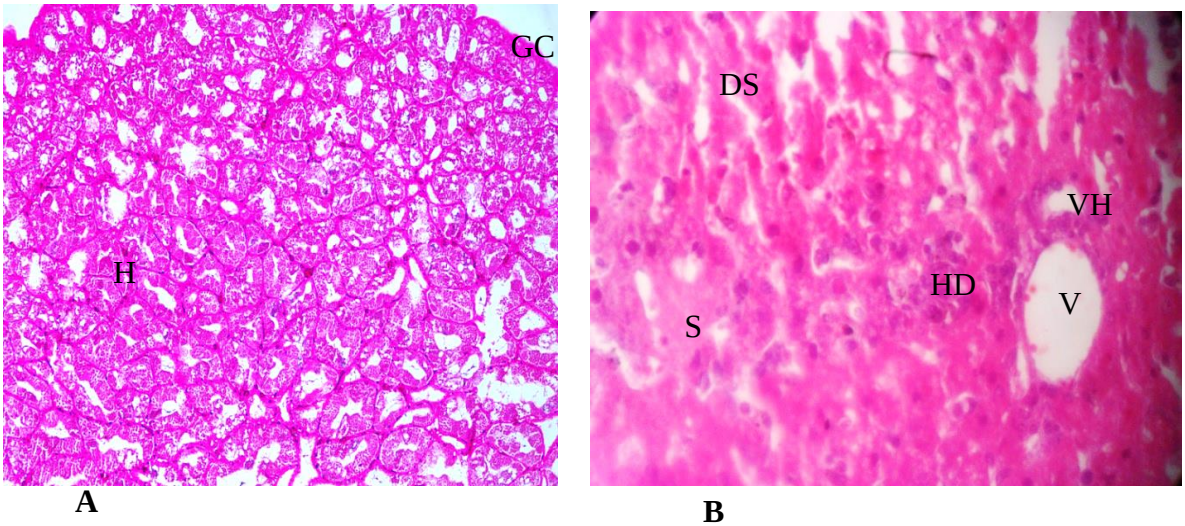

Figure 3 (A – B): *Oreochromis mossambicus* liver showing the effect of *C. papaya* on the hepatic tissues. A represents normal hepatic tissue of the group fed BD with no inclusion of PSM, and B represents hepatic tissue from fish fed 30g PSM/ kg of BD. H – hepatocytes; S – sinusoid; GC – Glisson's capsule; V – hepatic venule; VH – vacuolization of hepatocytes; HD – hepatic degeneration; DS – dilation of sinusoids.

Discussion

The male:female ratio 1.09:1 achieved for the negative control group (P0M4) that received the BD in this study did not deviate significantly from the expected 1:1 (M: F) ratio. The diets that contained PSM resulted in the masculinization of the treated animals, which was in agreement with the observation of Ampofo-Yeboah (2013). The percentage of males increased with an increase in the PSM content of the diets. The maximum percentage of sex reversed fish (77.8%) differed considerably from a 65% masculinization rate that was reported by Ampofo-Yeboah (2013) for *O. mossambicus* treated with pawpaw seed powder. Pawpaw seeds contain some phytochemicals such as fatty acids, crude proteins, crude fiber, papaya oil, carpaine, benzyl isothiocyanate, benzyglucosinate, flavonoids, glucotropacolin, benzylthiourea, hentriacontane, saponins and β-sitosterol (Krishna et al. 2008). Estrogenic phytochemicals can exhibit anti-estrogenic tendencies in the presence of endogenous estrogens (Clotfelter and Rodriguez 2006). The observed change in sex ratio in this study may be attributed to the interaction of phytoestrogens present in pawpaw seeds with endogenous hormones of tilapia. This suggests that the dietary inclusion of PSM could affect gonadal sex differentiation in fish. Extending the treatments for 120 days did not improve the M:F ratio compared with those after 30 days of treatment. This can be ascribed to the fact that sex reversal takes place during the period of sex differentiation, which occurs between 10–20 days post-hatch in *O. mossambicus* (Varadaraj and Pandian 1987). Results from this study indicated that a prolonged treatment with PSM exceeding the period of sex differentiation did not result in a higher percentage of males obtained. In tilapia species masculinization occurs 7–37 days post-hatch (Kwon et al. 2000).

Different techniques have been employed in the production of mono-sex male tilapia, the most popular of which is the use of steroid hormones to skew the sex ratio in favor of males. The hormone 17α-methyl testosterone (MT) is the hormone of choice to stimulate masculinization of tilapia species (Babiak et al. 2012). In this study, the positive control which consisted of 60 mg MT/kg BD, resulted in 83.3% males and 16.7% females including those with damaged gonads, i.e. a male: female ratio of 5:1. The males with testicular lesions and females with ovarian lesions were considered as functional males and females respectively since gonadal pathology induced by the PSM is reversible upon withdrawal of treatment. In practical tilapia farming, sex reversal of less than 95% may not be ideal since as little as 5% females in a culture facility can reproduce indiscriminately to the extent of literally stunting cultures. Water temperature is the most effective environmental factor in sex reversal (Baroiller and D'Cotta, 2001). High temperatures decrease aromatase activity, thereby predisposing to masculinization (Tzchori et al. 2004). High temperatures also enhance the intake of the treated feeds and with it the quantity of the ingested hormone. In the present study, the physico-chemical parameters recorded during the experimental period were within the tolerable limit for tilapia culture. However, the water temperature in the present study was apparently suboptimal to effectively allow for a complete sex reversal to take place. If the water temperature of the experimental system was higher than 24.48±1.46°C recorded here, it could have contributed positively to the masculinization process in this study.

The relationship between fish weight and length (i.e. condition factor) frequently used, compared the effect of biotic and abiotic factors on the health or well-being of fish did not differ statistically between the treatments. Even though there were significant differences in weight and other morphometric parameters observed between some treatment groups, no dose response relationship was established in the present study. The fact that the sex reversed fish in both the PSM and MT treated groups did not demonstrate any growth advantage over the untreated groups may be due to the short culture period of the experiment. At prolonged nursing of 4 to 6 months, mono-sex tilapia attain larger individual size than their mixed sex counterparts (Little et al. 2003). A prolonged culture period may present an economic advantage for the tilapia aquaculture industry if growth differences in relation to sex are fully exploited. The

interest in the aquaculture industry for the production of mono-sex culture especially tilapia species was borne out of the assumption that male fish have a growth advantage over their female counterparts. Apart from the growth advantage ascribed to male tilapia, mono-sex culture is also practiced to mitigate the impact of indiscriminate spawning prevalent in the mixed culture system which predisposes to production of variable sized fish at harvest. Reduction of size variation at harvest enhances processing efficiency and economic value of the fish (Goudie et al. 1994). The respective treatment diets did not affect survivability of the treated fish and were also not related to the dose or duration of the treatment. The fact that treatment P30M1 had the lowest survival rate was probably due to minor toxicity of the pawpaw seed meal when given in higher doses especially during the fry stage of the fish.

The inclusion of PSM in the treatment diets resulted in moderate changes in the architecture of the reproductive and hepatic organs. The pathologies observed in the ovaries included fragmentation of the ovarian nuclei, hyperplasia of the follicular epithelium, and atresia of the follicles. The pathology which though mild was observed in fish fed high dose of the phytoestrogen. The mild alterations in the architecture of the gonads were reversible on discontinuation of the treatment with PSM therefore do not alter the functional M:F ratio obtained in this study. In the testis, the changes observed included hypertrophy of the interstitial cells, and mild vacuolation of the germ cells. Atretic follicles in the ovary and increased interstitial cells and focal necrosis of testicular tissue in Nile tilapia (*O. niloticus*) were observed (Jegede and Fagbenro, 2008). Similarly disintegrated sperm cells in Nile tilapia fed 4.9 g PSM /kg feed/day were reported (Ekanem and Okoronkwo, 2003). The histopathological lesions observed in the gonads in this study demonstrated that PSM, when administered at a dose higher than the optimum level required for masculinization and at a prolonged duration, can predispose the sex reversed fish to infertility which may be reversible on discontinuation of the treatment with PSM.

Mild alteration in the normal architecture of the liver was also observed in this study that included dilation of sinusoids, vacuolization of hepatocytes, and general hepatic degeneration. There have been few reports of changes in the architecture of the liver caused by additives in fish diets (Velisek et al. 2009). Though the pathology observed in the liver in the present study were mild, it indicates that at a dose higher than the 30 g/kg of BD, PSM may predispose to liver damage which will invariably lead to poor egg quality and composition since liver synthesizes yolk precursor vitellogenin. The fact that these lesions are minor and observed mainly in treatments fed high dose of the PSM for a prolonged period (i.e. 120 days) indicated a potential reversal of the phytoestrogenic effect of PSM on liver integrity after the withdrawal of treatment.

Conclusions

This study suggests that an inclusion level of 20 g PSM/kg BD is the optimal dosage to result in masculinization in *O. mossambicus*, with a 77.8% male population achieved. At this inclusion level the growth and survival of the fish were not negatively affected, and the minor histopathological lesions observed in the fish that received the higher doses and for a prolonged period, indicated that PSM was well tolerated. From the result of the study, it can be inferred that PSM as masculinizing agent may provide an alternative to 17α-methyl testosterone used in food fish production, thereby minimizing the influence of anabolic steroids on fish consumers, fish culturists, and on the environment.

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