Research Article

Comparative Studies of the Gonad Structure of Oreochromis Niloticus (GIFT) Fed with Pawpaw Seed Meal

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This study examines the impact of pawpaw seed meal using various level of inclusion in regulating procreation in Oreochromis niloticus (gift strain) via morphology (percentage defective sperm cells) as well as history of gonads using biomarkers. The pawpaw seed powder was compounded to a 2mm tilapia feed at 35% crude protein at 2, 4 and 6g per kg diet. Twenty (20) fingerlings (10 males and 10 females) with average weight of 29-32g were randomly allotted in triplicate into concrete ponds (1m x 1m x 1 0.8m) of 600 litres capacity with control. The Tilapia fish was nourished at 4% body weight per day in dual portions at 09.00 – 09.30 hours and 17.00 – 17.30 hours. The experiment lasted for 60 days. Minimal damage was done to the tissues of the fish testes as well as ovaries at lesser dietary PSM levels (2g per kg diet) whereas disintegration of a lot of cells occurred at increased dietary levels of PSM (4g and 6g per kg diet) which resulted to lack of spermatids and oocytes in the fish testes and ovaries respectively. Hence, it is useful in battling difficulties of over-population of tilapia in fish pond. Aside infertility, there were no observable contrary effect from pawpaw seeds consumption. Therefore, safe usage of 6 g PSM per kg diet for forty-five (45) days is recommended for economic efficiency of fish farms. Further research is required on reducing the antinutrient substances (carpaine) in pawpaw seed so that it can be safely use as an additive in feeds.

Keywords: *Histology, Gonads, GIFT, PSM, Inclusion*

INTRODUCTION

In order to ease the difficulties of malnutrition and starvation due to growing population, culturing of fish becomes an effective tool since fish is a valuable protein source alongside with several other qualities. Fish contains wet body weight around 16-20% protein when related to 3.5% in milk, 6.6% in rice as well as wheat and 12% in egg. It has great nourishing values with good sense of taste and about 85-95% higher digestibility rate (Anuar et al., 2008). Fish protein signifies a vital nutritive element in some countries that are densely populated where entire levels of intake of protein are very little (Food and Agriculture Organization, (FAO), 2004). According to FAO (2015), the rapidly developing sector which has surpassed population development is termed aquaculture.

Among the most significant set of farmed or cultured fish, tilapia is the second next to carp; in addition, it is the most extensively grown of any fish cultured on the earth (FAO, 2010). Moreover, Burden (2014) opines that Tilapia is cultured in no less than 85 nations of the globe. Tilapia is labelled ''aquatic chicken'' since it can be farmed commercially in an extensive variety of methods starting from the simple backyard methods to extremely intensive method or "factory farm" just like in poultry (Pullin, 1985). In majority of developing nations where tilapias are often cultured in cages, ponds, raceways, concrete tanks, as well as rice field are as a result of its characteristics which makes it appropriate for culturing (Fagbenro, 2002).

Tilapia reproduces naturally in the wild; based on this feature, it influences overcrowding in the systems of fish pond as well as reduced weight during harvest. Determination to alleviate this limitation include culturing of all-males which is mono-sex by means of exogenous hormone for sex reversal of sexually the same fish, predator usage, cage/tank culture, sterilization, high stocking density, sporadic or selective cropping, and usage of slow growing tilapia species (Mair et al, 1991; Beardmore, 1996; Fagbenro, 2002). Regardless of all the techniques examined into, hormone initiation of population of monosex appears to be the most encouraging and satisfactory method (Pandian & Varadarai, 2005). Fish sex hormonal control is achieved by applying a precise hormone to the fry prior to occurrence of sexual differentiation. Tilapia gonadal differentiation seems to take place within 8-25 days' post-hatch (Nakamura & Iwahashi, 1982). However, usage of hormones has shortcomings of costly technology, hatchery amenities as well as skilled workers are essential; likewise, hormones are costly and challenging to acquire (Jegede & Fagbenro, 2008).

On the other hand, there is need to investigate less costly, suitable technology which does not pose a risk to human and environmental health in solving the difficulties of uncontrolled tilapia breeding. The botanical name of pawpaw is Carica papaya and it has been discovered that pawpaw seed meal (PSM) consist of phytochemicals which holds boundless potential being sex reversal, likewise a procreative obstruction agent in culturing of fish. Hence, this study was carried out to compare the effects of the different inclusion eves of PSM on gonads of GIFT Tilapia.

METHODOLOGY

Study Area

This study was conducted at Durante Fish Industries Limited, Old Niger West Building, Challenge, Ibadan; South Western Nigeria.

Preparation of Experimental Diets

Ripe fruits of pawpaw, Carica papaya (East West Seeds Cinta breed) of honey dew diversity was gotten from Durante fish industries farm and dissected in order to take out the seeds afterwards sundried then pulverized into finely particle sizes of less than 250 µm. The particles were preserved in a dry, sterile container; 2mm skretting tilapia fish feed pellets were obtained from Durante Fish Industries limited with nutrient composition of 33% crude protein as well as 18.5MJ gross energy per kg feed was formulated as prepared diet by adding 2.0 g, 4.0g, 6.0g dosage treatment of PSM separately to 1 kg of 2mm pellet skretting tilapia fish feed.

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Table 1: Proximate composition of trial diets

Parameters	Values (%)
crude protein	35.23
crude fat	12.01
ash content	15.07
moisture	8.13
Fiber	8.26
Carbohydrate	21.3

SOURCE AND COLLECTION OF TEST FISH

A total of two hundred and forty (240) fingerlings of Oreochromis niloticus GIFT strain, obtained from a single spawn were collected from Durante Fish Industries Limited, Old Niger West Building, Challenge, Ibadan; South Western Nigeria. They were acclimatized for a period of 14 days in the concrete tanks and the fish were nourished with a profitable feed. After acclimatization, a total of 20 fish (10 males and 10 females) of average weight of 20g were stocked in 12concrete tanks (1m x 1m x 1 0.8m) each which was supplied with fresh water of 600 litres (temperature of water 27oC; hydrogen ion concentration (pH) 7.3; dissolved oxygen (DO) which ranges from 5.6-7.4 mg/l). The experimental units were duplicated thrice (3); in addition, the fish was nourished at 4% body weight daily in two portions at 09.00 to 09.30hrs and 17.00 to 17.30hrs for period of 45 days; afterwards they were separated, grouped by sex then weighed.

EXPERIMENTAL DESIGN

For experimental set-up, a total of twelve (12) concrete ponds (1m x 1m x 1 0.8m) of 600 litres capacity each was used for three (3) treatments, replicated three (3) times with control and replicates. Twenty (20) fingerlings (10 males and 10 females) with average weight of 29-32g were randomly allotted to each pond and filled with 500 litres of borehole water. The ponds were labelled, control (0g), 2g, 4g and 6g respectively.

HISTOPATHOLOGICAL STUDIES

Samples from kidneys, testis and livers were placed in formalin-saline solution in ratio 1:1 of 10% formalin plus 0.9% of sodium chloride for 24 hours. According to Luna (1992), histological segments of 5µ diameter was prepared conforming to the standard procedures.

STATISTICAL ANALYSIS

The collected data were accurately analysed via one-way Analysis of variance (ANOVA) as well as Duncan New Multiple Range (DNMR) post hoc test and mean differences at $p < 0.05$ were significantly considered.

RESULTS AND DISCUSSIONS

Histology of testes [Magnification 100X]

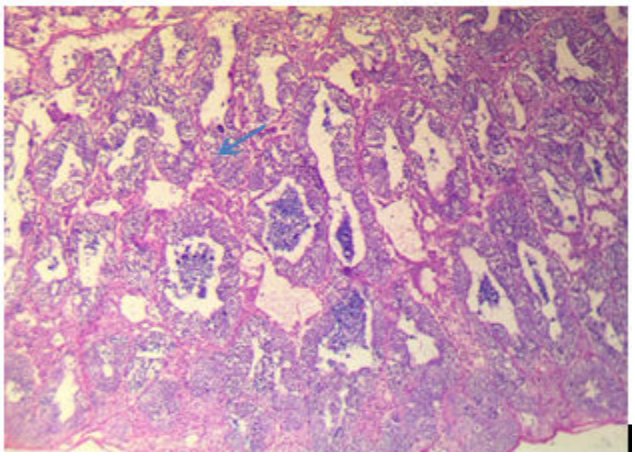

Plate 1: Histological section through the Testis (control).

0g PSM: The seminiferous tubules are numerous and contain fairly numerous amounts of spermatogenic cells (arrows). No visible lesion.

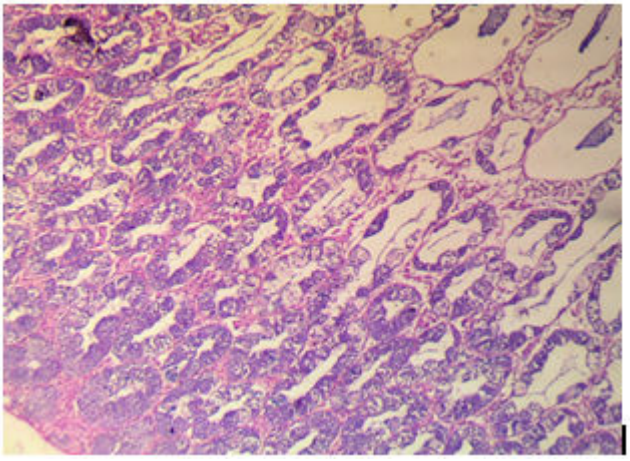

Plate 2: Histological section through the Testis (2g)

2g PSM: There are numerous seminiferous tubules which contain mildly depleted amounts of spermatogenic cells (arrows)

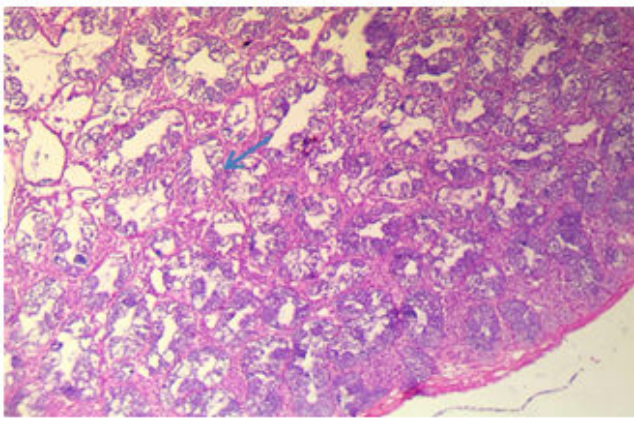

Plate 3: Histological section through the Testis (4g)

4g PSM: There are numerous seminiferous tubules which contain moderately depleted amounts of spermatogenic cells (arrows).

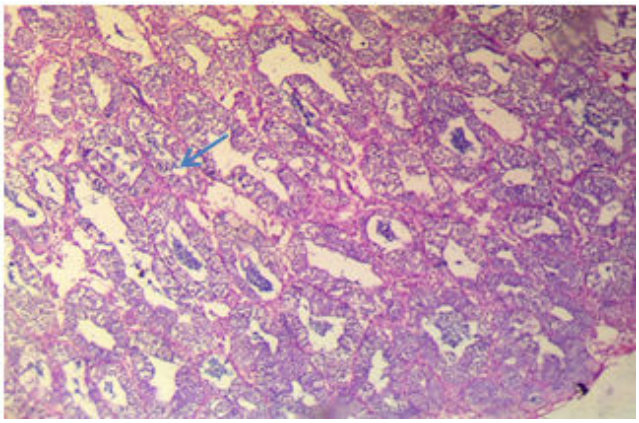

Plate 4: Histological section through the Testis (6g)

6g PSM: There are numerous seminiferous tubules which contain mildly depleted amounts of spermatogenic cells (arrows).

Plate 1 shows the histological unit of the testes in Oreochromis.niloticus which was nurtured with control diet of 0g PSM per kg revealed typical testicular tissue, numerous seminiferous tubules containing fairly numerous amounts of spermatogenic cells as well as normal distribution of sperm cell. There is observation of visible lesions. In Plate 2, tilapia fed with 2g PSM per kg diet indicated that there were numerous seminiferous tubules which containing mildly exhausted quantities of spermatogenic cells. However, the nuclei of the sperm cells were bloated, bigger seminiferous tubules which contain discreetly depleted quantities of spermatogenic cells were identified in the fish fed with 4g PSM per kg diet and 6g PSM per kg diet in Plate 3 and 4.

Findings revealed that there is a severe mutilation which was executed on the tissues of the testes as the PSM inclusion levels increases. This results were confirmed by the work of Ekanem and Okoronkwo, (2003) who examined the use of seeds of Carica papaya as a productiveness control agent for the male of O. niloticus and follow similar concentrations (60g, 120g/kg). They observed inflamed sperm cells nuclei of the fish fed with little dose while the high dosage of seed of C. papaya led to sperm cells disintegration in addition to the formation of extra inflamed sperm cells nuclei. Presence of seminiferous tubules in testis O. niloticus fed C. papaya was recorded by Temitope and Oyedapo, (2008). Furthermore, Temitope (2010) and Temitope (2011) observed similar histological effect in testis of Oreochromis niloticus which was treated with leaf meal of Hibiscus rosa sinensis as well as Aloe Vera Latex. Likewise, Feng (2011) observed severe deterioration and self-digestion of seminiferous tubules after Oreochromis niloticus was fed with 2g of pawpaw seed powder/kg diet for the period of 15 and 30 days.

Sperm Properties of Oreochromis niloticus

Table 2 and 3 indicates the results for sperm morphology and sperm progressive motility of Oreochromis niloticus fed various levels of inclusion of PSM. Concerning the various levels/weights, significant impacts were not observed on sperm motility, sperm count and sperm live/dead among all inclusion levels. Although, highest value of sperm motility, sperm count and sperm live/dead were recorded in "4g" PSM/kg diet while lowest values for sperm motility as well as sperm live/dead were reported in 0g while lowest value for sperm count was found in 2g respectively. Likewise, plate 1-4 indicated the histopathological study of testes which showed numerous seminiferous tubules containing depleted amounts of spermatogenic cells. This could be the cause of low sperm count as well as the high proportion of flawed sperm cells observed in 2g-6g PSM compared to the control groups. Similarly, unspecified amount of toxic elements and unfavourable consequence of the plants were reported. Furthermore, Seigler et al. (2002) presented (2R)-prunasin a small quantity of sambunigrin which is the major cyanogenic glycoside in Carica papaya whereas Wickersham and Novak, (2003) stated that there is a substance named carpine which is toxic in nature which exist in pawpaw black seeds in trace amount. According to Nwaehujor et al. (2014), carpine has been discovered to lead to paralysis in large amounts, and also reduce pulse speed and weaken the nervous system. Moreover, Oyekunle and Omope, (2010) specified that the precise device through which the elements lessen sperm count is unidentified, nevertheless suggestions was made that the multiple papain might cross the barrier of the blood testes to apply dangerous impacts on seminiferous tubules control of the testes. A lot of studies have stated the existence of proteins in the sperm and semen. Additionally, prior study recounted the occurrence of serum proteins (lactoferrin, albumin) and glycoproteins (kinase, prostatic). The function of these proteins are to nurture the sperm cells. In 4g and 6g PSM, the amplified fraction of flawed sperm cell might be as a result of action of proteolytic of the proteases in pawpaw chymopapain and papain or in addition to the steroidal glycosides (Edwards et al., 1981). There is possibility that the semen proteins have been hydrolyzed by enzymes and compounds which makes them unobtainable for usage of the sperm cells, thus resulting to undernourishment plus flaws perceived in the cells.

Table 2: Sperm Morphology of Oreochromis niloticus fed various inclusion levels of Pawpaw Seed meal

Parameters	0g	2g	4g	6g	S.E	F-Statistis	P-Value
Motility	73.33 a	83.33 a	86.67 a	80.00 a	7.93	1.944	0.201
Live / Dead	92.67 a	96.00 a	97.00 a	96.00 a	3.6	0.777	0.539
Count	194.67 a	193.00 a	208.33 a	204.33 a	13.81	0.827	0.515

Source: Field Survey and SPSS Output, 2018

Means in the same row having same small letters are not significantly different at 1% and 5% respectively

S.E = Standard Error

P-value = Probability Value

Table 3: Sperm progressive motility of O. niloticus fed various inclusion level of Pawpaw Seed meal

Parameters	0g	2g	4g	6g	S.E	F-Statistics	P-Value
Curved Tail	3,248.33 a	3,212.67 a	3,212.00 a	2,921.33 a	795.31	0.083	0.968
Looped Tail	11.00 a	11.00 a	11.00 a	11.00 a	8.528	0	1
Curved Mid Piece	3,879.00 a	2,879.00 a	2,879.00 a	3,948.33 a	842.73	1.869	0.231
Bent Mid Piece	3,252.00 a	3,251.33 a	3,581.67 a	2,882.00 a	888.39	0.247	0.861
Tailless Head	248.00 a	151.33 a	184.67 a	151.33 a	106.11	0.474	0.709
Headless Tail	14.33 a	248.00 a	148.33 a	184.33 a	122.92	2.969	0.097
Rudiments Tail	11.00 a	11.00 a	11.00 a	11.00 a	8.528	0	1
Bent Tail	3,882.33 a	3,582.00 a	3,215.33 a	2,915.00 a	845.51	0.684	0.586
Total	806.67b	805.00 b	808.33 b	462.67 a	155.81	926.97***	0

Source: Field Survey and SPSS Output, 2018

Means in the same row having different small letters are significantly different at 1% and 5% respectively

S.E = Standard Error

P-value = Probability Value

Study by Bucholtz et al. (2008) opine that development of gonad is an incessant procedure, nonetheless particular histological features could be used during reproductive stage to categorize phases of gonadal growth. Therefore, all these histological modifications in ovary and testis of the trial fish are as a result of dietary levels of PSM. The current findings were in line to elucidate the achieved outcomes in both sexes (male as well as female) Nile tilapia (Ekanem & Okoronkwo, 2003; Jegede & Fagbenro, 2008; Abbas & Abbass, 2011). This study used dosage of 2 to 8g per kg with period of exposure of 45 days which contrasts from the usage of prior studies. Likewise, Jegede (2009) and Jegede (2010) reported that Aloe vera latex as well as leaf of Hibiscus rosa-sinensis which are medicinal herbs cause related changes in the ovaries and testis of Nile tilapia; and likewise on laboratory animals like rats as well as rabbits (Lohiya et al., 1999; Goyal et al., 2010). Moreover, Farnsworth et al. (1975) opined that 5-hydroxytryptamine is a lively substance which is accountable for the anti-implantation result of seed of papaya in female. Also, papaya seeds extracts are competent of creating functional abnormalities of various mammalian organs/tissues plus systems possibly as a result of toxic impacts of benzyl-isothiocyanate. Additionally, Lucidi et al. (2003) stated that active growth of oocyte could influence steroidogenesis. Also, histological units which consist of atretic cavities might be as a result of decline in the level of oestrogen (Khalil et al., 2014). findings in this study revealed that the acquired outcomes indicated that PSM high stages of 4 and 6 g per kg on extensive periods of exposure for 45 days in feeds of Nile tilapia fish showed positive influence to control the procreative procedure in Oreochromis niloticus which caused numerous histological fluctuations in the testis which abridged fertility in males Nile tilapia.

CONCLUSION AND RECOMMENDATION

The study revealed that the damage which has been done on the tissues of the ovaries and testes was negligible at decreased dietary level of PSM (2g per kg diet), and greater dietary levels of PSM (4g and 6g per kg diet) caused breakdown of a lot of cells and rendered the testes lack of spermatids. Based on this, dry seeds of pawpaw are recommended for use in controlling tilapia breeding. Histological examination of tilapia testes nourished with basal feed which is enhanced with PSM indicated that seeds of pawpaw are an efficient agent of inducing sterility as they were destructive to ovary and testes tissues. Hence, it is suitable in contending difficulties of overpopulation of tilapia in ponds. Other than infertility, there were no observable opposing reactions from the pawpaw seeds consumption. Moreso, further research is required on reducing the antinutrient substances (carpaine) in pawpaw seed so that it can be safely use as an additive in feeds.

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