Original Research Article

GONAD HISTOLOGY, PROXIMATE COMPOSITION AND GROWTH PERFORMANCE OF NILE TILAPIA FED WITH PAWPAW *(Carica papaya)* SEEDS POWDER

ABSTRACT

The study was carried out to determine the effect of different inclusion levels of pawpaw seeds powder (PSP) on the proximate composition, growth and histological structure Nile tilapia gonads. Nile tilapia were treated with pawpaw seeds powder at four levels at 0g, 4g, 8g and 12 g PSP/kg feed for 60 days. The proximate composition of the carcass of the Nile tilapia showed that the 8g PSP/kg feed treatment group had the highest values (mean ± SE) of Crude protein and ash (53.97 ± 0.094 and 20.05 ± 0.35) respectively. The highest body weight gain and specific growth rate was achieved at the 8g PSP/kg treatment level ~~(18.160 and 4.921383 respectively)~~, but this treatment level showed the lowest feed conversion ratio. Histology of gonads of Nile tilapia treated with different levels of PSP revealed that ovaries and testes of 0g PSP/kg feed were normal. Ovaries of the 4g PSP/kg feed had degenerative stromas while testes had scanty spermatozoa. At 8g PSP/kg feed, the ovaries showed increased atretic follicles and testes had degeneration of spermatozoa. Treatment with 12g PSP/kg feed resulted in severe atretic follicles of the ovaries and deformation of seminiferous tubules and erosion of spermatozoa of the testes. The results of this study showed that pawpaw seeds powder can be used to control the breeding of Nile tilapia in production units.

Keywords: Histology, Proximate composition, Growth, Pawpaw seeds powder, Nile tilapia.

1. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) grows fast and is a widely cultured species with distribution in both the tropics and temperate regions. It is the most dominant fish among the tilapias that are farmed in sub-saharan Africa, and it is ranked second in global production after carps [1], [2]. Tilapia display many suitable characteristics as a culture species including general hardness, high disease resistance, high harvest level, growing on many natural and artificial foods as well as good consumer acceptance in the market [3]. Additionally, the species also withstand low concentration of oxygen, harsh ecological conditions, overcrowding, and tolerate different salinity levels [4]. Spawning occurs naturally throughout the year and reach sexual maturity at an early age of 2 – 3 months under normal circumstances [5]. Tilapia farming in Kenya if well developed, can provide the needed fish protein as well as monetary gains to fish farmers [6].

In the recent years, medicinal herbs and plants have been used as additives in fish feeds due to their various favourable and economically reflected results. Nowadays, most chemical materials and substances used in fish feeds have been banned due to health concerns. This has led to concentration on the use of organic materials in fish feeds. Pawpaw (*Carica papaya*) is available all year round in the tropics. It has several active components which include carpine, chymopapain, papain, aglycone of glucotropaeolin, benzyl isothiocyanate, a glycoside sinigrin, myrosin, and carpasemine. Pawpaw, particularly the seeds contains antifertility properties [7]. Various researchers have used pawpaw seeds to inhibit reproduction in *O. niloticus* [8], [9], [10] [11] and [12]. Most of these studies used pawpaw seeds to inhibit reproduction in

adult *O. niloticus* after sexual maturation. The studies reported that the larval stages of *O. niloticus* contain both the ovarian and testicular tissues, and sexual differentiation starts shortly after hatching or after the first feeding starts.
[11] reported that high PSP levels of 6 and 8 g PSP/kg feed on 45 to 60 days exposure period in the feed of *O. niloticus* after hatching were effective in controlling the reproduction in *O. niloticus.* This was evident by the decreased levels of testosterone and progesterone sex hormones and the histological alternations in testes and ovaries, which reduced the fertility of *O. niloticus*. [9] reported significant success after using pawpaw seeds powder in the feeds of male O*. niloticus* to induce sterility. [11] reported that the dietary PSP at the level of 6 g/ kg feed for a period of 45 days after yolk sac absorption of *O. niloticus* fry may be used to promote growth of *O. niloticus*. The administration of PSP in the diet of the *O. niloticus* improved several growth performance parameters which included survival, food conversion ratio (FCR) and fish body composition. On the contrary, [9], [12] and [13] reported that adding PSP in fish diets caused delayed growth and high mortality in *O. niloticus* fry.
The effectiveness as well as harmfulness of *C. papaya* in fish have however not been well studied. Hence this study was carried out to determine the histological effects, proximate body composition as well as growth performance of *O. niloticus* fed with different levels of PSP.

2. MATERIALS AND METHODS

2.1 Study Area

The study was carried out in the University of Eldoret fish hatchery in the Department of Fisheries and Aquatic Sciences for 60 days. The university is located in Uasin Gishu County, 9 Km north east of Eldoret Municipality, on the Eldoret - Ziwa road in Rift valley, Kenya. It lies 0°35°N and 35°N-12°E at an altitude of 2180M above sea level. Temperatures ranges from 8.4 °C - 25 °C, with bimodial rainy seasons ranging between 900mm to 1,200mm per annum.

2.2 administration of pawpaw seeds powder

A commercial diet of 45% Crude Protein and size 0.5mm was locally purchased from Jambo fish farm in Mumias, Kenya. Four different inclusion levels of pawpaw seeds powder in *O. niloticus* feeds were administered at 0g, 4g, 8g and 12g PSP/kg feed and feeding started one day after yolk sac absorption for a period of 60 days. Fry were fed at 10% body weight/day in four instalments daily at 0900h, 1100h, 1300h and 1500h, and feeding rate was adjusted to 8%, 6% and 4% body weight/day depending on fish growth for 60 days.

2.3 Histological examination of the gonads

Histological examination of gonads was conducted at the Eldoret Pathology Diagnostics Laboratory. Fifteen fish from each treatment were dissected by using a scapel to cut from the genital papilla to the pectoral fin, then opening a window on the side and removing the viscera leaving gonads in place. The gonads were then removed using a forceps and placed in an embedding cassete then immediately dipped in 10% buffered formalin for histological examination. The gonads were processed according to standard histological techniques described by [14]. Fixed tissues were then dehydrated with 30%, 70%, 90% and absolute ethanol, embedded in paraffin wax and sectioned at 8 µm using a microtome. Sectioned tissues were then dewaxed and dehydrated a second time with 30%, 70%, 90% and absolute ethanol and stained with Hematoxylin and Eosin (H&E). The tissues were then mounted and gonadal structures were studied and identified under a light microscope (mag ×40). Sex identification was based upon the existence of oocytes in the females and upon the lobular morphology of the testes in the males. Prepared sections were viewed under the microscope to observe the structure of the seminiferous tubules with spermatocytes and ovaries with oocytes so as to determine the effect of PSP on the structure of ovaries and testes.

2.4 Proximate analysis of *O. niloticus*

Analysis of crude lipids, Dry Matter (DM), crude protein (CP) and ash contents of the control and experimental *O. niloticus* treated with PSP at the end of the 60 days of the experiment were conducted according to the method of [15]. Ten fish from each aquarium were collected and oven-dried for proximate composition determination as follows: DM, by weighing differences before and after oven drying at 105 ^{0}C until a constant weight was obtained; crude lipids, by using ether for Soxhlet extraction; ash, by incinerating at 550 ^{0}C for 8 hours in a muffle furnace; and CP, by the Kjeldahl method after acid digestion to obtain Total Nitrogen (TN), and a factor of 6.25 was used for converting TN to CP of the fish. ~~The following formulas were used to calculate the above contents:~~

~~$$DM = \frac{\text{Weight after drying}}{\text{Weight before drying}} \times 100$$~~

~~$$CP = Total\ Nitrogen\ Concentration \times 6.25$$~~

$$\text{Crude Lipid} = \frac{\text{Weight of sample} - \text{Fat free residue}}{\text{DM (g)}} \times 100$$

$$\text{Ash} = \frac{\text{Ash (in grams)}}{\text{DM (g)}} \times 100$$

2.5 Determination of fish growth

Fish growth performance was measured by using the parameters of Body Weight Gain (BWG), Specific Growth Rate (SGR) and Food Conversion Ratio (FCR). The following formulas were used to calculate the stated growth parameters:

$$Body\ Weight\ Gain\ (BWG) = \frac{Final\ Weight\ (g) - Initial\ Weight\ (g)}{Initial\ Weight\ (g)}$$

$$Specific\ Growth\ Rate\ (SGR) = \left\{\frac{[Ln\ (Final\ Weight) - Ln\ (Initial\ Weight)]}{Time\ interval\ in\ days}\right\} \times 100$$

$$Food\ Conversion\ Ratio\ (FCR) = \frac{Weight\ of\ dry\ feed}{Final\ Weight - Initial\ Weight}$$

Where; *Ln* is the natural log.

2.6 Statistical data analysis

MINITAB (version 17.0) software was used for statistical analysis to calculate means and standard error. Descriptive statistics (Mean ± SE) was used for distinguishing differences in growth performance parameters and proximate composition of O*. niloticus* fed on different levels of pawpaw seeds powder.

3. RESULTS AND DISCUSSION

3.1 Histological structure of gonads of *O. niloticus* treated with different levels of pawpaw seeds powder

Photomicrographs of histological sections of the ovaries and testes of *O. niloticus* treated with different PSP levels are presented in plates 1-4. The results revealed visible histological effect of feeding *O. niloticus* with PSP on the structure of gonads. Ovary histology revealed normal yolk droplet and oocyte development and distribution in the treatment fed 0g PSP/kg feed (plate 1 a). Yolk droplet, oocytes and ovary follicles were observed in the treatment fed 4g PSP/kg feed. However, this treatment showed degenerated ovary stromas (plate 2 c). Treatment with 8g PSP/kg feed resulted in increased atretic follicles of the ovary (plate 3 e) and treatment group at the level of 12g PSP/kg feed revealed severe atretic follicles of the ovary (plate 4 g). The ovary follicles of the 8g PSP/kg feed and 12g PSP/kg feed were degenerated. Testes histology of the 0g PSP/kg feed group showed normality with primary and secondary spermatozyte, connective tissue and normal spermatozoa (plate 1 b). Testes of the group fed 4g PSP/kg feed had evidence of some degree of normality with presence of spermatid, spermatocytes and connective tissues. However, the testes in this treatment group had scanty spermatozoa (plate 2 d). Treatment with PSP beyond 4g PSP/kg feeding level showed abnormalities of the testes whereby the testes of *O. niloticus* fed 8g PSP/kg feed showed degeneration of spermatozoa in the ductus deference (plate 3 f) and those fed 12 g PSP/kg feed having deformed seminiferous lobules and severe erosion of the spermatozoa (plate 4 h).

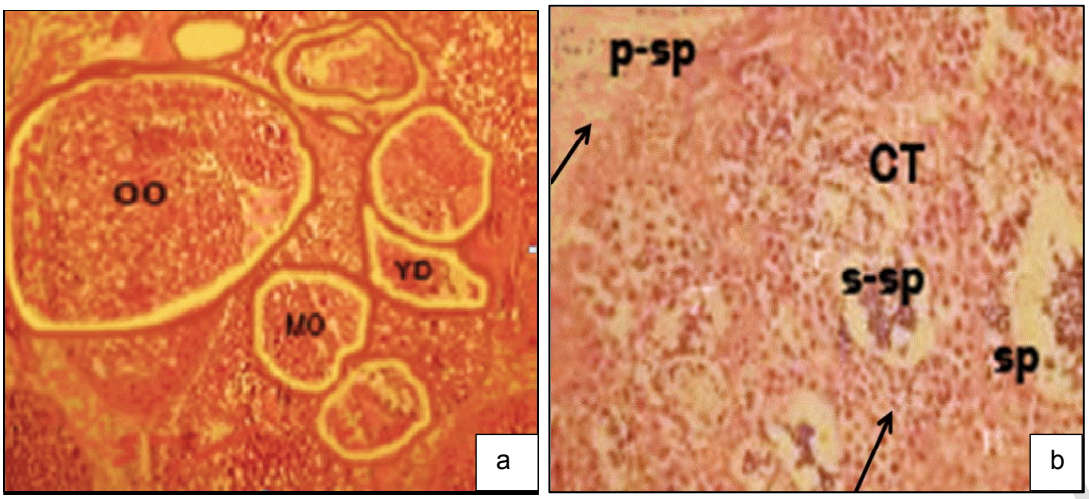

Plate 1: Photomicrograph of histological sections of *O. niloticus* ovary fed PSP/kg feed showing normal oocytes (OO) mature oocytes (MO) and a yolk droplet (YD) (a); and testes fed 0g PSP/kg feed showing presence of primary spermatocytes (P-SP), secondary spermatocyte (S-SP), connective tissue (CT), and normal spermatozoa (SP) (b). (Hematoxylin &Eosin stain, Mag. ×40)

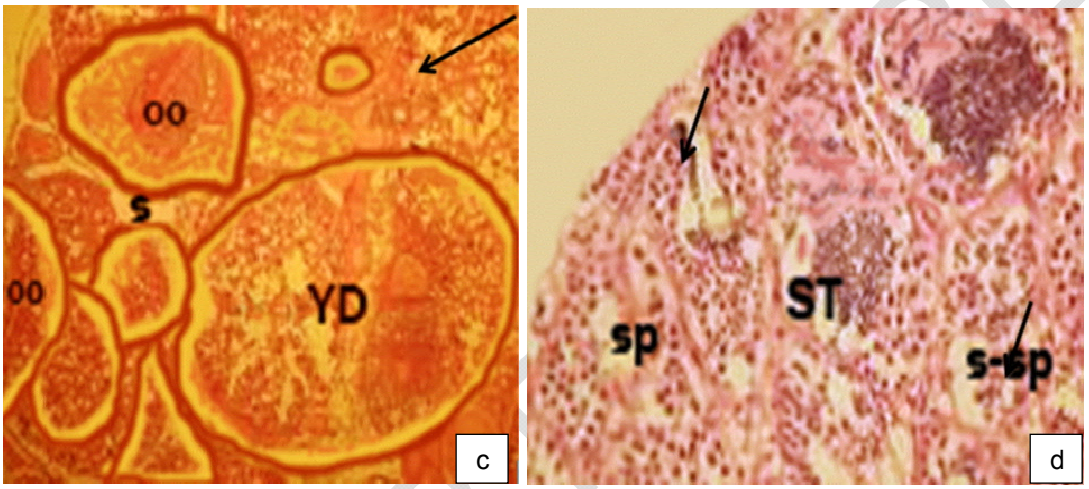

Plate 2: Photomicrograph of histological sections of *O. niloticus* ovary fed 4g PSP/kg feed showing mature yolk droplet (YD), developing follicle (arrow), degenerated stroma (S) and oocyte (OO) (c); and testes fed 4g PSP/kg feed showing presence of spermatocytes (SP), spermatid (ST) and scanty spermatozoa (S-SP) (d). (Hematoxylin &Eosin stain, Mag. ×40)

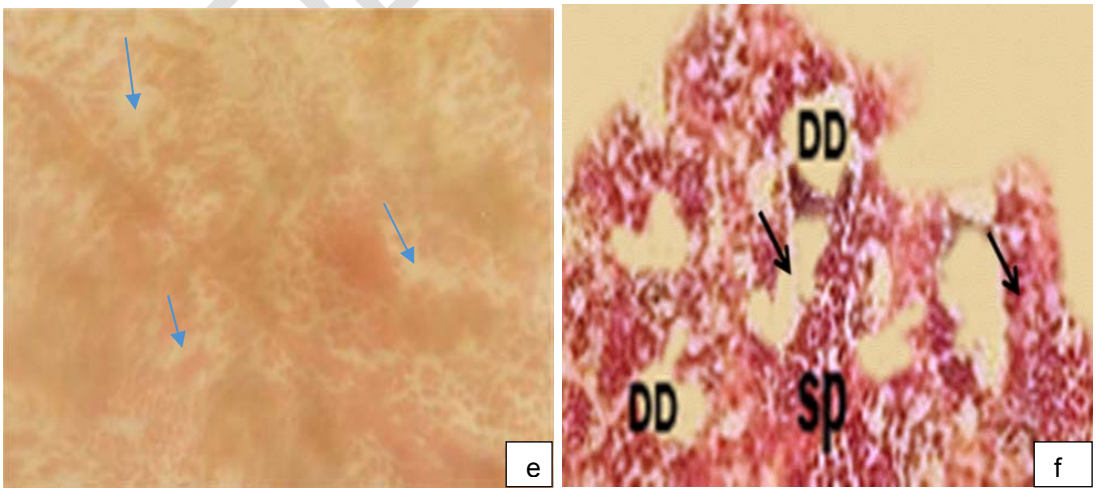

Plate 3: photomicrograph of ovary of *O. niloticus* fed 8g psp/kg feed showing increased atretic follicles (arrows) (e); and testes fed 8g PSP/kg feed showing secondary spermatocyte (SP) and degenerated spermatozoa in the ductus deference (DD) (f). (Hematoxylin &Eosin stain, Mag. ×40).

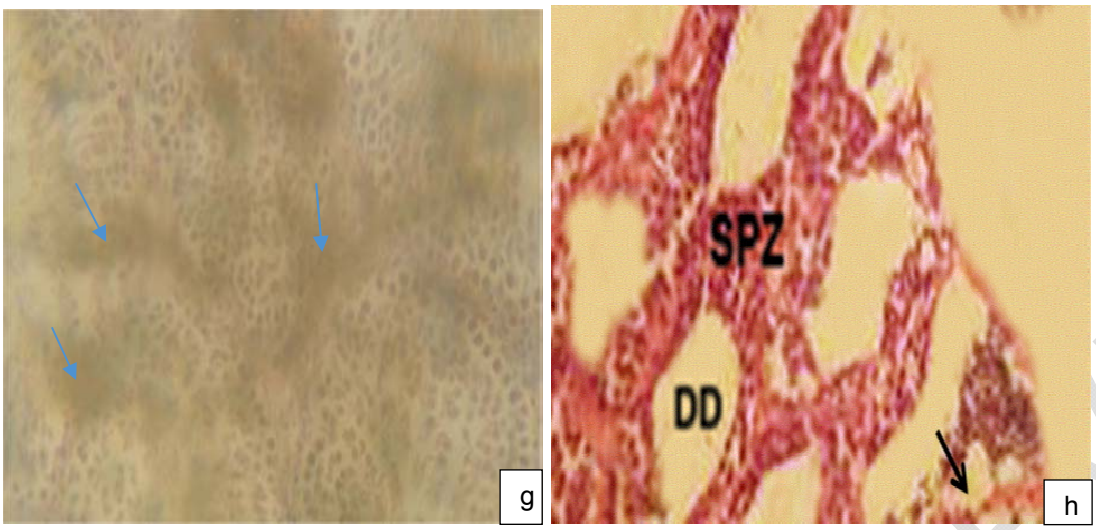

Plate 4: Photomicrograph of ovary of *O. niloticus* fed 12g PSP/kg feed showing severe atretic follicle (arrows) (g); and testes fed 12g PSP/kg feed showing high degeneration of spermatozoa in the ductus deference (DD) and severely eroded spermatozoa (SPZ) (h). (Hematoxylin & Eosin stain, Mag. ×40)

Different levels of PSP resulted in different intensities of abnormalities in the structure of both male and female gonads of *O. niloticus*. The quantities of pawpaw seeds powder used in this study (0-12 g PSPkg feed) is noticeably less than those used by [9]. In this study, 0 g PSP/kg feed treatment level had no effect on the gonads of *O. niloticus* while 4g PSP/kg feed treatment level caused minimal damage to the testes and ovaries. Higher PSP quantities at 8g PSP/kg feed and 12g PSP/kg feed on the other hand resulted in the degeneration of numerous cells, with the testes and ovaries lacking spermatids and oocytes, respectively. Degenerative stromas were observed in ovaries of females treated with 4g/kg PSP. [10] reported that *Tilapia zilli* fed with 2g/kg neem leaves (*Azadirachta indica*) had necrotic ovaries. Comparable results were reported by [16] who fed hibiscus leaves to *O. niloticus* at 3g/kg feed and [17] who administered *Aloe vera* latex at 2ml/kg feed and found similar histological changes to this study. [18] reported that pawpaw seeds have compounds that disrupt the endocrine system leading to reduced fish reproduction by either affecting gonad differentiation or by delaying gonad maturation. This agrees with the results of the current study which found degenerated stroma in ovaries and scanty spermatozoa in testes in the group fed 4g PSP/kg feed. Histological sections of the testes in all treatment groups except the control revealed either degenerated, eroded or scanty spermatozoa and deformation in the ductus deference. The level of deformities observed in this study increased with levels of PSP inclusion in the feeds of *O. niloticus*. Comparable similar results were obtained by [19] who reported continuous mild stroma degeneration in ovaries of juvenile *O. niloticus* fed 2g/kg feed; deformed seminiferous lobules and degenerated spermatozoa in the testes with increasing level of pawpaw seed meal from 2g/kg to 8g/kg feed. Relatively similar findings were also reported by [20] who included pawpaw seeds in the diet of mature *O. niloticus* at 120g/kg feed. The authors further stated that the substantial histological changes of the gonads in groups treated with high quantities of pawpaw seeds were permanent while medium and low quantities of pawpaw seeds may have reversible effects.

The findings of this study also matches well with the findings of [18] who reported that pawpaw seeds have active ingredients that may cause hypertrophy, hyperplasia, degenerated germ cells and germinal epithelium in both the testes and ovaries of fish. More prominent effects of pawpaw seeds on the histological structure of gonads were reported by [8], [9], [10] and [12] who studied the effect of pawpaw seeds on mature *O. niloticus* and observed severe hypertrophy in ovaries and very severe erosion and degeneration of spermatozoa in the testes. This study was done in the fry stage and the histological variations observed were less severe and less prominent than those reported by [8], [9], [10] and [12]. This may be explained by the exposure periods in the current study at fry stage which coincided with the critical period of gonadal differentiation which, according to [21] , is estimated to occur between 7 – 28 days post hatching in tilapia. The cited literature was treated at post-juvenile stage long after gonadal development had taken place.

3.2 Proximate composition of *O. niloticus* fed different levels of pawpaw seeds powder

Proximate composition of *O. niloticus* fed different levels of PSP at the end of the 60 day experimental period is shown in table 1. Crude protein content increased with increasing PSP levels upto the 8g PSP treatment level with the 8g PSP/kg feed treatment level showing the highest value (mean ± SE) for crude protein (53.97 ± 0.094) while the 0g PSP/kg feed had the least value for crude protein (51.25 ± 0.250). Crude lipid content on the other hand decreased with increasing PSP levels upto the 8g PSP/kg feed, where the highest crude lipid content was recorded in the 0g PSP/kg feed (17.38 ± 1.175) and lowest in the 8g PSP/kg feed (13.9 ± 0.450). Beyond the 8g PSP/kg feed, the crude lipid level increased again as recorded in the 12g PSP/kg feed treatment group (14.75 ± 0.200). Ash content was highest in the 8g PSP/kg feed treatment level (20.05 ± 0.35) and lowest in the 0g PSP/kg feed treatment level (18.00 ± 0.250). DM was highest in the 0g PSP/kg feed (3.88 ± 0.004).

Table 1. Proximate composition (Mean ± SE) on dry matter basis of *O. niloticus* fed different PSP levels for 60 days

Proximate composition (%)	Treatment			
	0g PSP	**4g PSP**	**8g PSP**	**12g PSP**
Crude Protein (CP)	51.25 ± 0.250[c]	52.88 ± 0.063[b]	53.97 ± 0.094[a]	53.19 ± 0.188[a]
Crude Lipid	17.38 ± 1.175[a]	16.18 ± 1.575[a]	13.9 ± 0.450[c]	14.75 ± 0.200[b]
Ash content	18.00 ± 0.250[b]	19.10 ± 0.637[b]	20.05 ± 0.35[a]	19.53 ± 0.23[a]
Dry Matter (DM)	3.88 ± 0.004[a]	3.86 ± 0.006[b]	3.86 ± 0.009[c]	3.86 ± 0.014[c]

Superscript in the same row sharing a common letter were not statistically different

This study showed that treatment with 8 g PSP/kg feed led to higher Crude protein (CP) and ash contents and lower crude lipid in the whole body composition compared to other treatment levels. These results did not agree with the study of [12] who reported that *O. niloticus* that were fed with 6 g PSP/kg/ day had lower Dry Matter, Crude Protein and crude lipids and increased moisture. Generally, the contradicting results with respect to the proximate composition of the fish in this and other studies may be explained by the different fish sizes used, different periods of exposure, and also the nutritional and physiological status of the fish used in the studies. This study showed that in order to achieve improvement in fish body composition, PSP could be incorporated in the feeds of the fish at an early stage immediately after hatching raising the level of pawpaw seeds powder to 8 g PSP/ kg feed for a long period of 60 days. Further studies however need to be conducted to investigate the effect of longer exposure periods of beyond 60 days on the proximate composition.

3.3 Growth performance parameters of *O. niloticus* fed different levels of pawpaw seeds powder

The results of the effect of different PSP levels on growth performance parameters of *O. niloticus* are shown in table 2. All growth performance parameters were statistically significant in all treatment levels (mean ± SE). The growth performance parameters progressively increased with increasing PSP levels upto the 8g PSP/kg feed treatment level, then decreased with increasing the PSP level to 12g PSP/kg feed. The highest values (mean ± SE) of all growth performance parameters were recorded at 8g PSP/kg diet treatment level except for FCR which was lowest at this treatment level (2.56 ± 0.012).

Table 2. Growth parameters of *O. niloticus* fed different levels of PSP during the study period

Parameters	Treatment			
	0g PSP	**4g PSP**	**8g PSP**	**12g PSP**
Initial weight (g)	0.22 ± 0.02[a]	0.22 ± 0.02[a]	0.22 ± 0.02[a]	0.22 ± 0.02[a]
Final weight (g)	3.70 ± 0.05[b]	3.61 ± 0.00[c]	4.13 ± 0.04[a]	3.48 ± 0.05[d]
Average weight gain (g)	3.48 ± 0.02[b]	3.39 ± 0.01[c]	3.91 ± 0.03[a]	3.26 ± 0.02[d]
BWG	16.15	15.73	18.16	15.13
SGR (%)	4.74	4.69	4.92	4.63
FCR	2.87 ± 0.09[c]	2.95 ± 0.02[b]	2.56±0.01[d]	3.07±0.01[a]

Superscript in the same row sharing a common letter were not statistically different

This study revealed positive effect of PSP on growth performance parameters of *O. niloticus*. The results of this study were contrary to those of [9] and [12] who reported that adding PSP to *O. niloticus* feeds for a treatment period of 30 days resulted in reduced growth during that period. [22] used Aloe vera latex in the feeds of *O. niloticus* and reported no significant differences in the growth performance parameters and Feed Conversion Ratio. [16] used *Hibiscus Rosa-sinensis* leaf meal in the diet of fish and reported good growth performance in *O. niloticus* fed the normal diet; while weight gain, average daily gain and specific growth rate on the other hand were reduced in fish fed the *Hibiscus Rosa-sinensis* leaf meal. The contrary results of the current study to other studies may be attributed to the different sizes of fish, different fish species in some cases, different PSP quantities used and different duration of exposure employed in the present and previous studies. This study used newly hatched fry of *O. niloticus* that were subjected to different levels of PSP (0, 4, 8 and 12 g PSP/kg feed) for 60 days, while the contrary were used in the studies of [9] and [12] where adult males of *O. niloticus* of average weight of 40g were fed with different levels of PSP (4.9 g/kg/ day and 9.8 g PSP/kg/day) for 30 only. Likewise, [10] administered different levels of PSP (0, 0.5, 1.0, 1.5 and 2 g PSP/kg diet) to *O. niloticus* of an average weight of 40g and for 60 days.

4. CONCLUSION

High PSP levels of 8g PSP/kg feed and 12g PSP/kg feed severely affected the gonadal structure of *O. niloticus*. This study therefore concluded that PSP in the feeds of *O. niloticus* is effective in controlling reproduction, and may be substituted with expensive and hazardous hormonal use to overcome problems of tilapia overpopulation in culture ponds. This study demonstrated the degenerative effect of PSP with increasing levels which may indicate that beyond an optimum level, gonads would be completely damaged and sterility could be induced. This optimum level of PSP and the effect of exposure for longer periods beyond 60 days could be the focus for future research. Pawpaw seeds can also be administered to *O. niloticus* in known amounts and duration in order to improve growth. However feeding fish on pawpaw seeds results in poor flesh quality as reflected in the high ash content in the 8g PSP treatment level (20.05 ± 0.35). Further studies should be done to investigate and quantify the occurrence of toxic substances in PSP.

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