Potency of plant hormone (*Carica Papaya* Seeds) on Growth Performance and Masculinisation of Tilapia 'Wesafu'

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Abstract
The production of tilapias remains low due to poor growth performance reported by fish farmers. One of the underlying reasons is the early female maturation at a very small size and precocious breeding in ponds, resulting in overpopulation which leads to stunted growth. This research investigated the potency of *Carica papaya* seed as a natural sex-reversal agent in tilapia production and its effect on growth. A total of 600 fry of Tilapia 'wesafu' were used; each weighing averagely, 0.47±0.01g. They were randomly distributed in triplicate into 12 hapas (1m×0.5m). Each hapa containing 50 fry, were placed in four earthen ponds (8m×3m). Fish in Treatment 1 were fed without (0.0g/Kg) powdered Pawpaw seeds which was the control. Fish in Treatments 2, 3 and 4 were fed with Pawpaw seed diet at doses of 15.0, 20.0 and 25.0g/kg feed respectively.
The result showed that fish fed at 20.0g/Kg and 25.0g/Kg of *C. papaya* had the highest final weight gain (20.92±0.29 g) and (21.13±0.29 g) at the end of the experiment respectively. The masculinisation revealed that T. 'wesafu' fed 20.0 g/Kg of *C. papaya* diet (81.67±0.85%) was higher and significantly different ($P<0.05$) compared to normal diet. Since natural hormones from plant materials (*C. Papaya*) are biodegradable in nature, it could be used as a viable alternative to synthetic hormones.

Keywords: Sex reversal, Tilapia, *Carica papaya*, growth
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Introduction

Tilapia 'wesafu' is a tropical fish species of the cichilidae family that is very prominent in Lagos Lagoon, by Epe, Lagos State. This Tilapia species is highly prized due to its large size fleshy body makng it a desirable food fish for culture (Fashina-Bombata *et al.,* 2005). It grows to about 1500g in body weight and with total length of about 414mm in the wild (Fashina-Bombata, *et al.,* 2005). The mature female radiates a golden colour when gravid and produces up to 1600 fry from one single spawn. Earlier studies have assumed T. 'wesafu' to be *Sarotherodon guineensis* which is believe to be abundant in the lagoon, but further studies on the morphometric characteristics shows T. 'wesafu' to be different from T. *guineensis* (Hammed *et al*., 2011). The fish is euryhaline and its nutritional qualities include 21% crude protein and 0.9% fat (Fashina-Bombata, *et al.,* 2005). They have the ability to reproduce under varying culture conditions and readily convert food low in plant protein to high quality flesh (El-Sayed, 2006). It has taxonomy features that distinguished it from other species of Tilapia, such as a distinct karyotype, with a different autosomal fundamental number and chromosome structure compared to Sarotherodon sp. T. 'wesafu is also larger with a deeper body than *Tilapia guineensis*, which it was previously mistaken for. Morphologically, it shows differences in head length, eye diameter, and body depth. It also has unique amino acid profiles in its tissues and blood compared to other Tilapia species (Fashina- Bombata and Megbowon, 2012).

Female of tilapia species have a high fecundity and reproduce very fast once they are of age even at a small size and exhibiting stunted somatic growth at higher densities while male tilapias like *Oreochromis niloticus* and *O. aureus* exhibit faster growth rates and are often the preferred gender for monosex aquaculture (Hines and Watts, 1995). With the purpose of achieving more productivity in growing Tilapia 'wesafu', it is important to produce mono-sex culture that constitutes totally of males (El-Greisy and El-Gamal, 2012). The use of non-synthetic or synthetic hormones (steroid) is one of the most efficient methods of producing all-males by feeding newly hatched fry with these homones mixed with their feed (Ajiboye *et al.,* 2015).

Masculinisation of *Oreochromis niloticus* (Nile Tilapia) has has been successful and adopted in many commercial hatcheries, but not much has been done to domesticate T 'wesafu' with interesting results obtained from a study conducted by Fashina-Bombata *et al.* (2008). The use of 17α methyltestosterone is the most common practice for many aquaculturist and it is more efficient and a relatively cheap means of masculinising tilapia fry (Phelps and Popma, 2000; El-Sayed, 2006). However, the increased use of these synthetic steroid hormones to produce monosex populations of tilapia for intensive productive systems may lead to environmental and public health concerns.

The use of non-synthetic chemical compound extracted from plant materials extracts containing bioactive principles such as alkaloids, flavonoids, pigments, phenolics, terpenoids, steroids and essential oils has been reported to promote various activities like antistress, growth promotion, appetite stimulation, tonic and immunostimulation, and antimicrobial properties in fish culture (Chakraborty and Hancz, 2011). Previous studies showed that *C. Papaya* seeds have been used as fertility control agents in some laboratory animals like rats (Udoh *et al*., 2005) and monkeys (Lohiya *et al*., 2002). Many researchers have also used *C. Papaya* (pawpaw) seeds powder as a natural reproductive inhibitor in Nile tilapia (Ekanem and Bassey, 2003; Jegede and Fagbenro, 2008; Abbas and Abbas, 2011). To our knowledge, this study is the first on the effect of dietary supplement of *C. papaya* on masculinisation of T. 'wesafu' Therefore, the study was undertaken to evaluate the effect of dietary supplementation of *C. papaya* seed powder on growth performances and masculinisation of T. 'wesafu' fry.

Methodology

Gravid females and males of T. 'wesafu' were collected from Iwopin (Lagos Lagoon, close to Epe), Ogun State and transported with oxygenated bags and stocked together for mating into hapa nets (1mx1m) in an earthen pond (8mx3m) at ratio 2:1 female and male respectively for spawning, fertilization and breeding. The eggs were checked from females by applying slight pressure on stomach toward the anus part. The fish is a mouth brooder, so the three days old fry were collected. 600 Tilapia 'wesafu' fry were used; each weighing 0.47±0.01 g. They were randomly distributed in triplicate into 12 hapas (size of 1m×1m×0.5m) of 50 fry. Four earthen ponds (8mx3m) were used. The hapas were suspended in an earthen pond.

Ripe fruit *Carica papaya* were procured and the seeds were extracted and washed in sterile distilled water, air-dried and milled into fine particle size (<250 µm) and kept in a dry, clean, air-tight container. Phytochemical properties present in seed powder were checked before adding to the formulated ration of 40% crude protein. The pawpaw seed (PS) were added to the compounded feed at different doses (0.0, 15.0, 20.0, and 25.0g/kg) and wetted with deionised water, mixed thoroughly, formed into pellets with a manual fabricated pelletiser machine (diameter 1mm), and air dried at room temperature. The pelleted feed was pulverized before feeding to the fish fry.

The three-day old T. 'wesafu'fry (0.47±0.01g) were batch-weighed and randomly stocked into four groups. Treatment 1 (T1) were fed without powdered Pawpaw seeds (control 0.0g/Kg). Fish in T 2, 3 and 4 were fed at doses of 15.0, 20.0, 25.0 g/kg respectively. Fish were fed three times for 30 days. Feeding rates were adjusted every 30 days for 120 days based on the weight gain of each group of fish.

Growth performances were assessed by determination of survival rate (SR), specific growth rate (SGR), weight gain (WG) and daily weight gain (DWG); using the following formulae:

$$\text{Survival rate (\%)} = \frac{\text{Number of experimental fish at the end of experiment}}{\text{Number of experimental fish at the beginning of experiment}}$$

SGR (%/day)= In final weight-In Initial weight/time(days)×100

WG = Mean final weight (g) – mean initial weight (g)

DWG (g/day) = mMean final weight (g) – mean initial weight (g)/ day.

Twenty individual fish per replicate were randomly selected for sex determination. The sexes were determined anatomically by examining their internal and external reproductive organ (Mohamed, 2015).

The design was a 1×1×4 factorial arraignment in a completely randomized design of one fish species (T. 'wesafu'), one test materials (*Carica papaya* seed) and four levels of test material supplementation (0.0, 15.0, 20.0 and 25.0 g/Kg).

Data collected were analysed with SAS Statistics Version 9.1 using one way analysis of variance (ANOVA) to determine the level of significance of the treatments and the differences between the means were separated at P<0.05 using Duncan multiple range test (DMRT). Descriptive analysis was used to determine the percentage males and females in each treatment and final weight against treatment.

Result

Growth performance

The growth and production parameters of T. 'wesafu' in the four treatments are shown in Table 1. Diet with *C. Papaya* at 25.0 g/Kg showed no different in the weight gain 21.13±0.29g compared to fish fed at 20.0 g/Kg (20.92±0.29g) but significantly higher than 15.0g/Kg (19.93±0.29g) and the control diet (13.84±0.29g) respectively. Observation on specific growth rate showed no significant increase in fish receiving diet containing *C. Papaya* at 25.0, 20.0 and 15.0g/Kg compared to fish fed with the control diet (0.0g/Kg). Fish fed diet contained *C. Papaya* at 25.0 and 20.0g/Kg showed no significant difference (P>0.05) but it was higher compared to fish fed with *C. Papaya* at 15.0g/Kg and 0.0g/Kg in the DGR. Specific growth rate (SGR) showed no significant different among fish fed at 25.0g/Kg, 20.0g/Kg and 15.0g/kg of *C. papaya* but were significant (P<0.05) higher than fish fed at 0.0g/kg. Fish fed with the highest dose of *C. papaya* (25.0g/Kg) showed significant (P<0.05) lower survival rate compared with other fish fed at lower doses.

Table 1: Growth performance of Tilapia 'wesafu'

(g)	0	15	20	25	SEM
Final	13.84^{d}	19.93^{c}	20.92^{ab}	21.13^{a}	0.29
Initial	0.47	0.47	0.47	0.47	0.01
MWG	13.37^{d}	19.46^{c}	20.46^{b}	20.66^{a}	0.29
SGR	2.83^{b}	3.14^{a}	3.18^{a}	3.19^{a}	0.02
DGR	0.11^{c}	0.16^{b}	0.17^{a}	0.17^{a}	0.00
Survival (%)	86.67^{a}	86.67^{a}	85.33^{a}	74.67^{b}	0.82

^{abc}Means with different superscript along the same row are significantly different (P<0.05)

MWG - Mean weight gain, **SGR** - specific growth rate and **DGR** - Daily growth rate

Masculinisation

Table 2 showed the masculinisation of T. 'wesafu' fed different levels of the *C. papaya.* Fish fed at 0.0 g/Kg had the lowest percentage of males (35.00%) and was significant (P<0.05) different compared with fish fed at 20.0 g/Kg had the highest percentage of males (81.67%). The highest percentage (65.00%) of female was obtained from T. 'wesafu' fed the controlled diet (0.0g/kg) which was significant (P<0.05) different from other Treatments.

Table 2: Masculinisation of T. wesafu

	0	15	20	25	SEM
Male (%)	35.00^{c}	75.00^{bc}	81.67^{a}	76.67^{b}	4.24
Female (%)	65.00^{a}	25.00^{b}	18.33^{c}	23.33^{bc}	4.24

^{abc}Means with different superscript along the same row are significantly different (P<0.05)

Discussion

Growth performance of T. 'wesafu' indicated that there was increase in the growth as the level of *C. papaya* meal was increased in the diet which is similar to the report by Piferrer and Donaldson (1991) for Coho Salmon *Oncorhynchus kisutch* and Nyadjeu *et al.* (2019) on use of *Gacinia kola* seeds supplemented diet on growth performance of *O. niloticus*. This research also agrees with Ugonna *et al.* (2018) who reported that as the level of inclusion of *C. papaya* seed meal in the diet increased, the growth of *O. niloticus* increased. This is contrary to the report by Goudie *et al.* (1994) and Little and Edwards (2004) who reported an insignificant effect of hormone treatment on growth of *Ictalurus punctatus* (Channel Catfish) and *O. niloticus* (Nile Tilapia) respectively. Phytogenic compounds had been shown to be a growth promoter in monogastric animals (Hafeez *et al.,* 2016), as it confirmed in this study with T. 'wesafu'. It was shown that some plants improve nutrient ultilisation for better growth in fish. Most probably fat was used for energy, and protein was used for growth in different plants diets. Although the anabolic function of the androgen has been implicated for better performance observed in previous studies. An increase in palatability of the diets caused by increased *C. papaya* inclusion could be the cause of the high growth performance observed in this study. Uneaten feed, which is an index of lowered palatability, was not observed from *C. papaya* treated groups compare with the control.

The growth enhancement achieved with natural plants containing phytoestrogenic effects has been demonstrated in many fishes, such as the *Cyprinus carpio* (Common Carp), *Oncorhynchus mykiss* (Rainbow Trout), *O. niloticus* (Nile Tilapia), *Oreochromis aureus* (Blue Tilapia), and *Perca flavescens* (Yellow Perch) (Malison *et al*., 1988; Jegede, 2012). Previous studies on plants hormones have rarely reported highest inclusions but have drawn their conclusions based on observed differences between treatment means (ANOVA). The optimal inclusion diet of *C. papaya* that gave the maximum weight gain (21.13g) in this study was 25.0 g/kg.

This study showed that the treatments did not affect the percentage survival of the fish, which agrees with the report of Ampofo-Yeboah (2013). However, Pandian and Varadaraj (1987) reported higher mortality in hormonal masculinisation of fish compared to the control group. Ridha and Lone (1990) established an inverse relationship between the dose of hormone and the survival rate of *Oreochromis spilurus* (Sabaki Tilapia), indicating that sex reversal pre-disposes fish to a lower survival rate. However, Jensen and Shelton (1979) reported a higher survival rate in hormonal sex-reversed fish than in the control. The findings of this study suggest that adding of 20.0 g/kg *C. papaya* meal to the diet of fish was not lethal to T. 'wesafu'. Omeje (2016) similarly proposed that non-lethal levels of phytochemicals in *C. papaya* seed meal were the reason for lower mortality observed in *Oreochromis mossambicus* (Mozambique Tilapia).

The inability of T. 'wesafu' to spawn could be attributed to hormones (steroid) in the plant (*C. papaya*) into the diet to alter the undetermined sex of tilapia fry to males. This is in line with the study of Ajiboye and Yakubu (2010) who reported that mono-sex culture method has been widely used to control the precocious maturity and uncontrolled reproduction in tilapias with the use of non-synthetic hormones from plants. Masculinisation in this study was highest in the diet which contained 20.0g/kg *C. papaya* (80% males). This is in agreement with Ugonna *et al.* (2018) who reported that as the level of *C. papaya* seed meal inclusion in the diet increased, it produced over 85% male *O. niloticus*.

Conclusion

The study revealed that the use of non-synthetic hormones extracts from *C. papaya* seed meal added into the feed fed to T. 'wesafu' fry for masculinisation showed no negative impact on the health status, growth performance and high mortality rate. C*arica papaya* seeds added at 20.0 g/Kg (81.13%) dose gave the best masculinisation percentage than other doses and significantly higher than fish fed the basal diet. The diets showed no high mortality rate at 0.0 g/Kg to 20.0g/Kg inclusion level of *C. papaya*. The study provide information on the use of organic hormones from *C. papaya* seeds to discourage tilapia fish farmers from using synthetic hormones which is expensive; thereby it will help to reduce the cost of Tilapia production.

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