GROWTH RESPONSE AND SURVIVAL OF NILE TILAPIA (*Oreochromis niloticus*) FED STEROID HORMONE AND PAWPAW SEED (*Carica papaya*) MEAL BASED DIET.

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ABSTRACT:
This study was conducted to evaluate the effect of wet testes of bull (Bos. indicus), boar (Sus. domesticus), combination of bull and mud catfish testes (B. indicus / C. gariepinus) and pawpaw seed powder (Carica papaya) on the growth and survival rate of O. niloticus fry in an indoor experimental ponds. Fifty five fry were assigned randomly to five experimental ponds in triplicate. The feed (composed of soya-bean, fish meal, wheat bran, cassava flour (garri)) in Pond one (G1), Pond two (G2), Pond three (G3), Pond four (G4), and Pond five (G5) was formulated by adding 17α-methyl-testosterone (0.06g), wet testes from bull (17.47g), boar (25.59g), combination of bull and catfish (20.48g) at a ratio of 80:20 and pawpaw seed powder (20g) respectively per kg of feed. The growth and survival rate of fry in G1, G2, G3, G4 and G5 was (3.59 ± 0.09^{c} & 72.33 ± 7.22^{b}, 4.00 ± 0.22^{bc} & 92.33 ± 5.78^{a}, 5.12 ± 0.09^{a} & 95.67 ± 1.45^{a}, 4.33 ± 0.41^{bc} & 97.00 ± 2.08^{a}, 4.70 ± 0.07^{ab} & 98.07 ± 3.03^{a}) respectively with significant ($p<0.05$) difference. From the result, it appears that the feed formulation in G3 was more favourable to the growth of tilapia compared to the formulation in G5, G4, G2 and G1 whereas the feed formulation in G5 had the highest survival rate.

Keyword: Aquaculture, feed composition, growth performance, food security.

INTRODUCTION

Aquaculture is considered as the fastest developing food-producing sector in the world; since it is a means for food stability, food security, conservation of species in danger of extinction and increase nutrition globally (Fečkaninová *et al* 2017). Its contribution to the world's prosperity and reducing pressure on wild fish stock cannot be overemphasized, as the world's capture fisheries have been exhausted due to over-fishing and anthropogenic activities. With the ever increasing human population, urbanization in developing states and expectation of growth in standard of living, demand for human food has become so intense (Tansey and Worsley, 2014). Hence to cope with this challenge, there is need to increase protein production to urgently resolve its demand. The swiftly increasing demand for organic food in the world market; has become a thought in the aquaculture of tilapia. Culture of fish is an efficient means to alleviate the problem of reduced fish meat (Béné *et al*., 2015).

Nile tilapia is a tropical specie native to Africa (Deines *et al*., 2014) with favourable characteristics, like tolerance to unfavourable environmental conditions (Edwards, 2015), survival at reduced dissolved oxygen level (Abdel-Tawwab *et al*., 2014), euryhaline (very tolerant to large change in salinity), high food conversion, fast growth (Sayed *et al*., 2015) and management conditions (Mortuza and Al-Misned, 2013). As a result of these characteristics, tilapia is currently one of the preferred fish for aquaculturist (Ferdous *et al*., 2013). The quality of Nile tilapia has a neutral taste and hard texture which makes it acceptable and preferred to other fishes (Madalla, 2008) Both females and males eat the same quantity of food, but males grow bigger than females; because of their ability of converting food to energy which makes its culture preferable to female (Mur, 2014). Hence, all-male is preferred in a production unit to prevent unwanted spawning. The various methods used in producing all-males tilapia are: Hormonal sex reversal, hand sexing, genetic manipulation and hybridization (Fuentes-Silva *et al.*, 2013). Furthermore, other methods are irregular harvest, biological control, stocking at high density use of chemicals (Fortes, 2005). Among the above listed methods used in aquaculture worldwide, hormonal sex reversal method is the most widely used, and it has been successful over the years in all-male tilapia production (Pandian and Sheela, 1995). However, skill labours, availability and hatchery facilities that are required to use this hormone might be difficult to obtain in some countries (Fortes, 2005). Therefore, there is a major hindrance of finding natural alternative and high growth rate for masculinization of tilapia.

In recent times, medicinal plants have been effectively used to stimulate sterility in laboratory animals (Khalil *et al*., 2014). Pawpaw seed powder have been used to induce fertility control agents in laboratory animals like rats (Udoh *et al*., 2005) and rabbits (Pathak *et al.*, 2001). Suliemen *et al.*, 2013, Phelps *et al*., 1996 and Odin and Bolivar 2011 fed with animal testes diet and observed high growth rate of Nile tilapia fry. Thus, this research focuses on growth and survival rate of Nile tilapia fed with formulated feed containing difference levels of steroid hormone, animal's testes and pawpaw seed, which are rather cheaper and accessible from local market and abattoirs within the country.

MATERIALS AND METHODS

The experiment was carried out in 15 different experimental ponds at the African Regional Aquaculture Center Aluu, (ARAC), Port Harcourt, Rivers State. Eight hundred and twenty five fry of *Oreochromis niloticus* (Nile tilapia) were assigned randomly into five groups, with Fifty five (55) fry each in triplicate using

15 concrete ponds while testes of boar, bull, 17α-methyl-testosterone matured African mud catfish and pawpaw fruit were procured and prepared as described by (Orose *et al.,* 2016). Five kilogram of feed was formulated based on the working composition obtained from the feed mill of ARAC, using 40% crude protein. The feed ingredients were composed of soya-bean, fish meal, wheat bran in various percentages as describe by (Orose, and Vincent-Akpu, 2016). The formulated feed and hormones were prepared by adding 0.06g of 17α-methyl-testosterone (G1), 17.47g of bull wet testes (G2), 25.59g of boar wet testes (G3), 20.48g of bull/ and mud catfish (G4) at (80:20) ration, 20g of pawpaw seed powder (G5) based diet respectively to 1kg of feed. Feeding was given at 20% of fish body weight for 42days hormonal trial, while for 4months the fry were fed with blood meal diet at 10% and 5% of fish body weight.

Procedure to Determine Biological Parameters

Growth performance was calculated according to the following formulae after Rashid (2010).

Mean Weight Gain (MWG)

Mean weight was recorded weekly using the formula below.

$$MWG = Wt_2 - Wt_1$$

Where: Wt_1 = Initial mean weight of fish at stocking (T_1)

Wt_2 = Finial mean weight of fish at the end of the experiment (T_2)

Total Feed Intake (TFI)

The total feed intake was calculated by the addition of weekly feed intake of fish in each group.

Feed Conversion Ratio (FCR)

This was calculated by dividing the amount of feed (feed intake) by weight.

$$FCR = \frac{total\ feeds\ consumed}{net\ weight\ of\ fish}$$ (Rashid, 2010)

Protein Efficiency ratio (PER)

PER was calculated as:

$$PER = \frac{mean\ weight\ gain}{mean\ crude\ protein\ fed}$$ (Rashid, 2010)

Where: mean CP fed = feed intake × % CP in diet

Specific Growth Rate (%SGR)

This was calculated as: SGR (% body wt.gain/day) $= \frac{\log e\ W2 - \log e\ W1}{T} \times 100$ (Rashid, 2010)

Where: T = trial duration (day)

W_2 and W_1 = mean final and initial weights (g), respectively

Estimation of Survival Rate

Survival percentage was done using the formula:

$$\%\ SUR = \frac{no\ of\ live\ fish}{total\ no\ of\ fish\ stocked} \times 100$$ (Rashid, 2010)

Condition factor (CF)

Conduction factor was calculated as:

$$CF = \frac{\text{weight gain}}{\text{total length l3}} \times 100$$ (Ariyaratne, 2011).

Proximate Analysis Procedure of Pelleted Feed

The proximate analysis for pelleted feed was determined on dry weight basis using the Association of Official Analytical Chemists (AOAC) method (2005). The parameters analysed for were crude protein, fat content, moisture, crude fiber, ash content and nitrogen free extract.

Data Analysis

The Results was analyzed using Statistical Package for Social Sciences (SPSS) version 21 to determine differences between group mean (±SE) using Duncan multiply range test (DMRT) at 5% level of probability and significant differences among the various group using One-Way analysis of variance (ANOVA).

RESULTS

Proximate composition of experimental diets

The results of the proximate composition of experimental diets are presented in Table 1. The crude protein content (CP) revealed that fry fed with boar testes (group 3) had the highest among the treatments (46.07±1.34%) while group 1 had the least crude protein content of 37.60±0.46%. There was significant differences ($p<0.05$) among all groups (Table 2). The moisture content of the experimental diets showed that group 4 was the highest with a mean value of (10.38±0.20%) while group 2 had the least moisture content (6.82±0.03%). group 3 and 4 had the highest ash content although with no significant ($p>0.05$) difference while group 1 showed the least ash content. The crude fibre content in group 4 gave the highest percentage (4.12±0.04) while group 1 had the lowest crude fibre content (1.20±020) group 5 showed the highest fat content in the experimental diets with mean of 7.79±005%. The least fat content was recorded in group 1 and 2 with no significant different ($p>0.05$).

Growth performance parameter at the hormonal period

The initial mean weights and total length of fry in groups 1 – 5 was not significantly different ($p>0.05$) as shown in table 2. The final mean weights for all groups were significantly different ($p<0.05$) (Table 2 and 3). Group I recorded the least mean final weight. There were significant differences ($p<0.05$) in the final weights, mean weight gain, total feed intake, feed conversion ratio, protein efficiency ratio, % specific growth rate. Fish fed with the group 1 diet showed ($p<0.05$) lower values for mean weight gain, total feed intake, protein efficiency rate, % survival and specific growth rate but had the highest feed conversion ratio. It was observed in this study that fish fed with group 3 diet had the highest mean weight gain, total feed intake, protein efficiency rate and specific growth rate. Group 4 had the highest % survival rate (Table 2). Protein efficiency ratio was highest in Group 2 (1.71±0.03). Group 3 recorded the least protein efficiency ratio. It was observed in this study that fish fed with boar testes group 3 had the highest mean weight gain, total feed intake, and specific growth rate. Group 3 had the highest % survival rate (Table 2).

Growth performance of fry fed with blood meal based diet

Growth performance of fry fed with blood meal based diet is presented in Table 3. The initial mean weights of fry in groups 1 – 5 were significantly different (p<0.05). Group 3 had the highest mean weight gain 10.96±0.45[a]). Final Mean weight was also (p<0.05). Group 3 had the highest final mean weight (11.76±0.46[a]) and group 1 had the least mean final weight gain (6.88±0.86[a.]).

There were significant differences (p<0.05) in the final weights, mean weight gain, total feed intake, feed conversion ratio, protein efficiency ratio, % specific growth rate and % survival. Fish fed with the group 1 diet showed (p<0.05) lower values for mean weight gain, total feed intake, % survival ratio, specific growth rate. Feed conversion ratio was highest in group 3 (2.21±0.04). Group 2 had the least feed conversion ratio 1.95±0.03 (Table 3).

Protein efficiency ratio was highest in Group 2 (1.71±0.03). Groups 3 recorded the least protein efficiency ratio. It was observed in this study that fish fed with boar testes group 3 had the highest mean weight gain, total feed intake, and specific growth rate. Group 3 had the highest %survival ratio of 96.84±1.65 and group 1 had the least survival ratio 85.86±4.61 (Table 3).

Table 1 Proximate composition of experimental diets

PARAMETERS	TREATMENTS				
%	G1	G2	G3	G4	G5
Ash	9.18±0.15^{c}	10.57±0.88^{b}	12.22±0.16^{a}	11.05.±0.37^{b}	12.73.±0.32^{a}
Crude Fat	5.10±0.05^{d}	5.08±0.72^{d}	6.21±0.06^{b}	7.79±0.05^{a}	5.50±0.17^{c}
Crude Fibre	1.20±0.19^{d}	1.83±0.20^{c}	3.01±0.07^{b}	2.74±0.25^{b}	4.12±0.04^{a}
Moisture	7.56±0.06^{b}	6.82±0.03^{d}	7.43±0.04^{bc}	10.38±0.20^{a}	7.14±0.05^{c}
Crude Protein	37.60±0.46^{d}	41.10±1.22^{bc}	46.07±1.34^{a}	42.30±0.59^{b}	39.20±0.30^{cd}
Nitrogen free extract	39.34±0.68^{a}	34.60±1.30^{b}	25.05±1.15^{d}	28.03±1.06^{cd}	29.01±0.44^{c}

Mean values (mean ± standard error) in the same row with different superscript are significantly different (p<0.0

Table 2: Growth performance parameters of sex reversed *O. niloticus* fry fed on five diets at the end of 42days experimental period

PARAMETER	TREATMENTS				
	G1	G2	G3	G4	G5
TIL(cm)	0.83±0.00^{a}	0.83±0.00^{a}	0.83±0.00^{a}	0.83±0.00^{a}	0.83±0.00^{a}
TFL(cm)	2.62±0.12^{b}	2.97±0.09^{ab}	3.38±0.19^{a}	3.07±0.29^{ab}	3.30±0.08^{a}
IMW(g)	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}	0.01±0.00^{a}
FMW(g)	0.42±0.02^{d}	0.52±0.05^{cd}	0.78±0.02^{a}	0.60±0.09^{bc}	0.69±0.02^{ab}
MWG(g)	0.41±0.04^{d}	0.51±0.05^{cd}	0.76±0.02^{a}	0.59±0.09^{bc}	0.68±0.00^{ab}
TMFI(g)	0.83±0.03^{b}	0.92±0.05^{b}	1.24±0.05^{a}	1.03±0.16^{ab}	1.21±0.03^{a}
FCR	2.04±0.05^{a}	1.81±0.08^{ab}	1.62±0.04^{c}	1.76±0.03^{b}	1.78±0.05^{b}
PER	1.23±0.03^{b}	1.38±0.06^{b}	1.55±0.04^{a}	1.42±0.04^{b}	1.41±0.06^{b}
SGR(%/day)	3.59±0.09^{c}	4.00±0.22^{bc}	5.12±0.09^{a}	4.33±0.41^{bc}	4.70±0.07^{ab}
SR (%)	72.33±7.22^{b}	92.33±5.78^{a}	95.67±1.45^{a}	97.00±2.08^{a}	98.07±3.03^{a}

Mean values (mean ± standard error) in the same row with different superscript are significantly different (p<0.05). ITL= Initial total length, FTL= Final total length, IMW= Initial mean weight, FMW= Final mean weight, MWG= Mean weight gain, TMFI= Total mean feed intake, FCR= Feed conversion ratio, PER= Protein efficiency ratio, SGR= Specific growth rate, SR= Survival rate

Table 3 Growth performance parameters of sex reversed *O. niloticus* fry at rearing experimental period fed with blood meal diet.

PARAMETERS	TREATMENTS				
	G1	G2	G3	G4	G5
IMW	0.42±0.02^{d}	0.52±0.05^{cd}	0.78±0.02^{a}	0.60±0.09^{bc}	0.69±0.02^{ab}
FMW	7.30±0.88^{b}	10.43±0.30^{a}	11.73±0.46^{a}	10.67±0.67^{a}	10.40±0.26^{a}
MWG	6.88±0.86^{b}	9.90±0.29^{a}	10.96±0.45^{a}	10.07±0.58^{a}	9.71±0.25^{a}
TMFI	15.37±1.87^{b}	19.53±0.31^{ab}	24.13±0.73^{a}	19.93±2.13^{ab}	22.00±0.77^{a}
FCR	2.24±0.10^{bc}	1.97±0.05^{c}	2.20±0.02^{a}	1.97±0.10^{bc}	2.27±0.02^{ab}
PER	1.49±0.07^{b}	1.69±0.04^{a}	1.51±0.02^{b}	1.70±0.09^{b}	1.47±0.01^{a}
%SR	85.86±4.61^{b}	93.51±2.61^{ab}	96.84±1.65^{a}	94.38±1.04^{ab}	94.44±1.07^{ab}

Mean values (mean ± standard error) in the same row with different superscript are significantly different (p<0.05)). IMW= Initial mean weight, FMW= Final mean weight, MWG= Mean weight gain, TMFI= Total mean feed intake, FCR= Feed conversion ratio, PER= Protein efficiency ratio, SGR= Specific growth rate, SR=Survival ratio

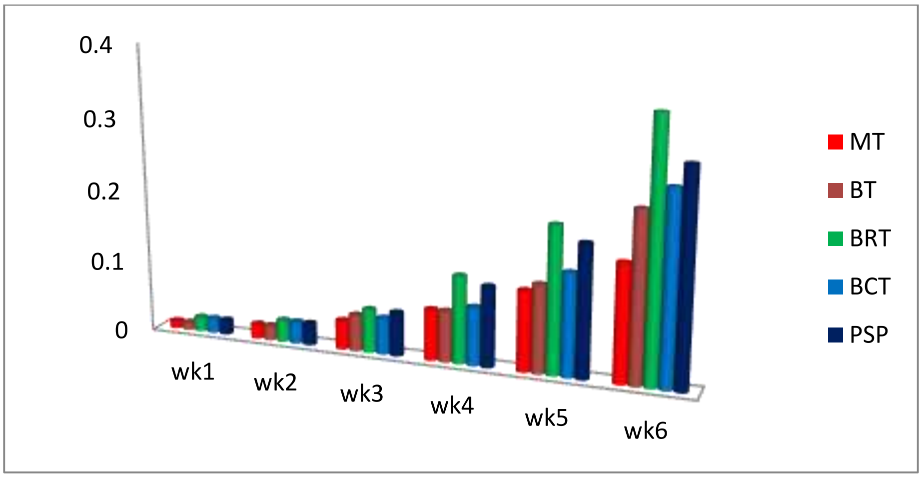

Figure 1: Weekly mean gain in weight of Nile tilapia (*Oreochromis niloticus*) fry during 42 days experimental trial

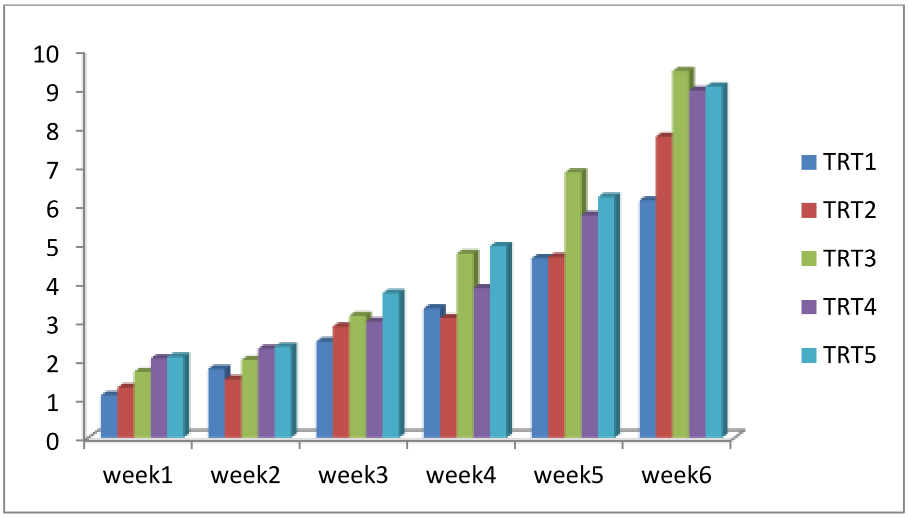

Figure 2: Weekly condition factor of Nile tilapia (*Oreochromis niloticus*) fry during 42 days experimental trial.

DISCUSSION

Chemical composition of experimental diets

The chemical composition of experimental diets showed that group III (boar testes) recorded the highest crude protein (CP). This is in line with the findings of Ahmed *et al*., 2004 and Al- Hafedh (1999) who observed 45 and 46% crude protein in animal testes diet compared to MT diet. Also Fashina –Bombata and Somotun (2008) reported similar CP in their diets with goat testes (47.33%). However, the CP in this study is a smaller than the report of Odin and Bolivar (2011). They observed CP level at 33- 71.69% among groups. The high CP might be as a result of the level of concentration of hormone found in the testosterone used. Furthermore, group 4 recorded a higher CP than the group 1 diet this could be as a result of crude protein and papaya oil found in pawpaw seed (Krishna *et al*., 2008). Group 5 had the highest crude fibre and ash content this might probably be due to high fibre in papaya as reported by Krishna *et al*., (2008) that *Carica papaya* has significant level of C. fibre. Moisture content was highest in group 4 it might be as a result of the 80:20% ratios of bull and catfish testes present in the composed diet.

Growth performance parameters

The weekly mean weight gain observed in this study was highest in group 3 and least in group I. SGR, FCR, PER, MWG were highest in group 3. This finding disagrees with Phelps *et al*., 1996, Odin and Bolivar 2011 who reported least growth performance of Nile tilapia fed with lyopolized testes from boar However, Odin *et al*., 2009 reported significant high growth rate in tilapia fed with dehydrated testes from carabao, cattle and hog fed for 23days. MT in this study had the least growth rate. Our findings in this study could be due to the fact that animal testes meal has higher protein which resulted in a high specific growth rate. Similarly, Odin and Bolivar 2011 reported the least growth rate with MT diet. Increase growth rate in group 3 could probably be due to increase in testosterone inclusion in the diet which increase the crude protein. Bhasin *et al*., 2001 reported that testosterone produces muscle hypertrophy by increasing muscle protein synthesis. In addition, Suliemen *et al*., 2013 fed lamb testicle meal to tilapia fry and observed an increase growth rate. Increase in FCR in group 3 1.52 is slightly inferior to FCR values reported by Sulieman *et al*., 2013 and Siddiqut *et al* 1991 using 34% CP. Group 1had the least weekly mean weight gain this might be as a result of the CP level and survival rate compared to other groups. Group 5 had a high growth this might be as a result of the uncontrolled appetite for the experimental diet observed in this study. During the rearing period, the mean weight gain in groups 2, 3, 4 and 5 where not significantly different from each other but significantly difference from group1. This shows that blood meal diet was effective. Although, at the start of the blood meal based diet experiment the weight gain of the different groups where not the same, but it was observed that at the hormonal trials, group one had a reduce growth rate as a result of their loss of appetite for experimental diets. Hence their high reversal rate as describe by Orose *et al*., 2016.

Survival Rate

The data on survival rate of Nile tilapia fry after the 4months experimental period are shown in table 2 .Group 3 recorded the highest survival rate of 96% , while groups 4, 5, and 2 which had a percentage of 94, 94 and 93 and was not significantly different from group 1 which had a survival rate of 85%. The high survival agrees with the findings of Odin and Bolivar 2011 who observed a high survival rate using carabao, bull and boar testes. White (2008) also obtained high survival rates (88-95%) of fry fed with animal testes. Amofo-Yeboah (2013) reported a higher survival rate of 96% using pawpaw seed. This might be due to Krishna *et al*., 2008 report that pawpaw seed contain some medicinal effect. The least survival of the group 1(MT) in this study is higher compared to Haylor and Pascual, 1991 who reported survival ratio of 44% after 80 days trial of *Oreochromis niloticus* fry that was fed with 17 alpha-methyl testosterone (40mg/kg diet).

CONCLUSION

From the findings of this study, the specific growth and survival rate of Nile tilapia after 42days experimental trial was higher in boar testes and pawpaw seed powder. These findings suggested the use of boar testes and pawpaw seed powder as an effective alternative to synthetic hormone (17α- methyl-testosterone), currently used in tilapia sex reversal. Hence, groundwork is required for further comparative studies on animal testes and plant extract to maintain growth and reversal rate. Based on the results of this study, future investigation should be to increase the inclusion level of natural hormone to have an effective dose that will give the best reversal rate. Feeding should be give at 2% to 5% per body weight to control the increase in growth rate of tilapia fry when using natural hormone to obtain an effective reversal rate.

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