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GROWTH PERFORMANCE AND NUTRIENT UTILIZATION OF NILE TILAPIA (*Oreochromis niloticus)* FED TOASTED FLAMBOYANT SEED MEAL (*Delonix regia)*

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ABSTRACT
A 8 weeks feeding trials was conducted to evaluate the suitability of toasted *Delonix regia* meal (TDRM) fed Nile tilapia *Orechromis niloticus* in a flow through system through their growth performance and nutrient utilization. Four Iso-nitrogenous diet were formulated to contain 35% crude protein and 9.5 % lipid. TDRM was included at different graded levels viz 0,10, 15, and 20 and were designated as D1 (0 % inclusion), D2 (10 % inclusion), D3 (15 % inclusion) and D4 (20 % inclusion). Each treatment had two replicates of 15 fish per tank, with an initial live mean weight of (1.19±0.03g). The results showed that fish fed D2 had the highest (P<0.05) growth performance; nutrient utilization and body composition than those fed with the other experimental diets while fish fed D4 had the lowest growth performance. However fish fed D1 and D3 were not significantly (P>0.05) different from each other. This study showed that toasted flamboyant seed meal would be a suitable ingredient for tilapia culture and can be used up to 15 % as an added ingredient in aquafeed production without any adverse effect on the fish.

KEYWORDS: *Delonix regia, growth performance, nutrient utilization, aquafeed. Toasted*

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INTRODUCTION

To sustain the unprecedented expansion in aquaculture sub sector, research and development of fish farming techniques is required, in order to obtain the most efficient, safe and cost effective methods for producing seafood in the aquaculture industry. One of the main concern for rearing finfish in a culture setting is the high cost of feed, and more importantly the heavy reliance on fishmeal and fish oil as the primary protein and energy sources in these feeds.

Fishmeal (FM) have become staple in aquaculture diets due to its nutritional benefits is very palatable and has exceptional nutritional value including an excellent balance of essential amino acids and essential fatty acids, which closely meet the requirements of most farmed fish, and also provides an excellent source of digestible energy and vitamins (Tacon, 1993). The high price of FM drives up the cost of fish production, and finding a relatively cheaper but equally effective and eco friendly alternative ingredient has become a seriouson-going research goal. Hence, there is interest in replacing FM with less expensive protein sources. Many studies have been conducted to evaluate the replacement of FM in practical diets for tilapia with cheap, locally available plant and animal protein sources (Novoa *et al.* 1997; El-Sayed 1998; Fasakin *et al.* 1999, 2005; Abdelghany 2003; El-Saidy and Gaber 2003; El-Saidy and Gaber 2004; Borgeson *et al.* 2006; Gaber 2006 Goda *et al.* 2007). Common legumes such as soybean meal, groundnut cake and cottonseed meal are in short supply in the tropics and often not available for sustainable animal production, hence, there is an attendant high cost of these ingredients (Fasakin 1999). Therefore the search for alternative ingredients that reduce feed costs and maintain adequate levels of growth and production can have a marked impact on the sustainability and profitability of aquaculture industry (Bake *et al.* 2009). The inclusion of this unconventional plant ingredient sources depend on other factors such as the nutrient content of the plant, processing conditions and economic feasibility (El-Sayed 1999).

Tilapia is the second most culture freshwater finfish in the world. Currently, farmed tilapia represents more than 75% of world tilapia production (FAO, 2009), and this contribution has been exponentially growing in recent years. Several factors have contributed to the rapid global growth of tilapia. Tilapias are easily cultured and highly adaptable to a wide range of environmental conditions. Tilapia feed on a wide variety of dietary sources, including phytoplankton, zooplanktons, larval fish, and detritus (Halver, 1989). Adult tilapia are principally herbivorous but readily adapt to complete commercial diets based on plant and animal protein sources.

Delonix regia a wild plant otherwise called (flame of the forest), originated from the continent of the America but found wild or as ornamental plants in various parts of the world including Nigeria (Purseglove, 1994). During the fruiting period, large quantities of seeds are produced in the numerous pods of each plant. Tonnes of this seeds are wasted every year since they are neither consumed by any animal nor utilized for any medicinal propose. Flamboyant seed meal compare favourably with mechanically extracted groundnut cake meal in terms of crude protein content (NRC, 1994). The crude protein content of mechanically extracted groundnut cake meal is approximately 40% (NRC, 1994). Grant *et al*. (1991) studied the haemagglutination activity of *D. regia* seed extract in rabbit, cattle, rat and human bloods and reported that the seed extract exhibited low haemagglutinin activity and contained non-toxic lectins. *D. regia* in spite of its potential to serve as a livestock feed and its relative abundance has remained largely unexploited and underutilized for animal nutrition. The need therefore arises to look into the nutritional potentials of various wild and un-conventional fruits and seeds abundantly present in our environments. Like other grain legumes, it contains some anti-nutritional factors which causes negative effects such as less efficient feed conversion, poor growth, intermittent scouring, wasting and sometimes death when consumed raw (Ologhobo *et al.*, 1992). Tannins, phytins, protease inhibitors, cyanogenic glycosides have been reported as various anti-nutritional inhibitors. Sorensen, *et. al*, 2009 Aletor and Fasuyi (1997) reported among other things that: tannin causes poor palatability and hence poor feed intake of diets containing high tannin content. On the account of the deleterious effect of feeding raw *delonia regia* to livestock, there is therefore the need for processing before usage. Various processing methods such as soaking, cooking, germination, potash-cooked have been used to for processing plant ingredients and reported Ologhobo and Fetuga, 1983, Ologhobo *et al.*, 1992 and Akinmutimi, 2001. The use of heat is known to have improved the digestibility of polysaccharides and metabolizable energy in addition to inactivation of trypsin inhibitors Fagbenro and Davis, (2003) associated with plant proteins. Toasting process is critical to denature and destroy antinutritional factors but not damage nutrient protein fractions, hence heat treatment via toasting to remove or reduce to lowest level antinutritional factors present in plant proteins.

Based on the foregoing, therefore the main objective of this research work was to evaluate and investigate the suitability of toasted *delonix regia* seed meal as an ingredient in the diet of Nile tilapia fingerlings *O.niloticus* through their growth performance nutrient utilization and body composition.

MATERIALS AND METHODS

Diet formulation and preparation

Flamboyant seed meal

Flamboyant pods were collected during the dry season from Centre for Preliminary and Extra Moral Studies (*CPES*) Federal University of Technology Minna, Niger State botanical garden. It was manually crushed to get the seed. The seed was processed by toasting at 80^{o}C for 60 minutes in a frying pan until the colour of the seed coat changed to golden brown. The seed was allowed to cool before grinding it into powder using hammer mill. The product was finally analyzed to determine Moisture, crude protein, lipid and ash contents as shown in Table 1

Soybean meal
Raw soybean was purchased from the Bosso market (Niger state). The soybean was processed by toasting the soybean in frying pan at 80^{o}C for 60 minutes to golden brown. It was analyzed as stated previously as shown in Table 1

Fishmeal
The fishmeal used in this experiment was obtained from the Makolo Fisheries store, along Keteren Gwari road Minna Niger state. The crude protein and lipid content of fishmeal were 61.25% and 11.94% respectively.
All the ingredients were separately milled and mixed with warm water to form consistent dough, which was then pelleted, sun-dried, packed in polyethylene bags and stored. The feed composition table is shown in Table 2

Experimental diets
Based on the nutritional requirements of tilapia (NRC 1993), four isonitrogenous and isolipid diets were formulated at 35 % protein and 9.5 % lipids, containing 10-30% toasted flamboyant seed meal at different levels of inclusion Table 2.

Experimental system and fish
The experimental fish, pure- bred *O. niloticus* fingerlings, with a initial mean weight of (1.19±0.03) were purchased from Tagwai Fisheries hatchery, Ministry of livestock and Fisheries, Minna, Niger state. The fish were transferred in a well-oxygenated water plastic container from hatchery to the Department of Water Resources, Aquaculture and Fisheries Technology laboratory, Federal University of Technology, Minna where the feeding trial was conducted. Upon arrival they were acclimatized in a transitional tank in the laboratory for four days and were fed commercial feed (coppense feed) at 35% crude protein once a day before the experiment commenced.

Fresh filtered dechlorinated tap water was supplied to the flow–through system, which consisted of 15-rounded plastic tank, each with an approximate capacity of 45 l at the flow rate of 250-300 ml/min. The water quality parameters in the system were monitored weekly, the temperature ranged between 26ºC-29ºC while the concentration of dissolved oxygen ranged between 5.94-7.82 mg/L and the pH values of the treatments ranged from 7.18-7.60. No critical values were detected for nitrite and nitrate.

Three replicates of each treatment using 20 fish per plastic tank were reared on each of the four diets. Feed was manually administered. The fish were fed three times daily at 5% of body weight at 0900, 1200 and 1600 hours. Feeding rate was subsequently adjusted according to their growth rates per plastic tank.

The uneaten and faecal matters were siphoned out of tank every morning before feeding, and 45 minutes after the fish have been fed. The fish were denied feed 24 h prior to sampling. Ten fish were randomly sampled on weekly basis, and weights were measured using a digital electronic weighing balance

Biochemical analysis
About 15 g initial sample and 10 g of final samples from each plastic tank were pooled separately and then homogenized using laboratory mortal and pestle. The major ingredient used for the diet; the formulated diet and the fish body samples were subjected to chemical analysis. Proximate composition analyses was determined according to AOAC procedures (2000). Moisture content was determined by drying samples at 105±2°C until a constant weight was obtained. Dried samples were used for determination of crude fat, protein and Ash contents. Crude fat was measured by solvent extraction method in a soxhlet system where n-hexane was used as solvent. Crude protein content was calculated by using nitrogen content obtained by Kjeldahl method. A conversion factor of 6.25 was used for calculation of protein content according to AOAC (2000).

Evaluation of growth parameters
Growth performance and diet nutrient were analyzed in terms of Weight Gain (WG), Feed Efficiency (FE), Specific Growth Rate (SGR), Feed Intake (FI), Protein Efficiency Ratio (PER) and Protein Retention (PR). The following formulas were used:

Weight gain (%) = (final weight (g) – initial weight (g) x 100 / initial weight (g)

Feed efficiency (%) = (weight gained (g) / feed fed (g)) x 100

Specific growth rate (%) = (In final weight (g) – In initial weight (g) / feeding period (day) x 100

Feed intake (mg/fish/day) = dry feed (mg) given / number of fish / feeding period (day)

Protein efficiency ratio = wet body gain x 100 / protein intake (g)

Protein retention (%) = protein gain x 100 / protein fed.

Statistical analyses
Data were analyzed using one – way analysis of variance (ANOVA) using statistica 6.0 (Stat-Soft, Inc., USA). Differences between treatments were compared by Tukey’s test. Level of significance was tested at $P<0.05$.

RESULTS
Table1 showed the proximate composition of the major ingredients used in formulating the experimental diets. Fishmeal as usual has the highest crude protein and lipid content (61.25 and 10.70), followed by soybean meal (43.07 and 7.0) while, the crude protein and lipid content of toasted *Delonix regia* was 22.00 and 10.30 respectively.

The proximate composition of the experimental diets (Table 4) showed that the crude protein of the diets were similar and ranged between 33.4 and 34.9 %. Similarly the lipid, ash and moisture content of the diets were close and ranged between 9.25 and 9.44 %, 9.94 and 10.63 %, and 5.18 and 5.44 % respectively.

It was observed that there was no feed rejection by the fish fed the experimental diets, and they vigorously ingested the experimental diets. The change in weight per week and growth performance data of Nile tilapia fingerlings fed the experimental diets for 56 days are summarized in Fig. 1 and Table 3. Table 3 showed that fish fed 10% inclusion of toasted Delonix regia meal (D2) had the highest values of all growth performances and was significantly different from the other treatments ($P<0.05$). However fish fed 15% inclusion of toasted *Delonix regia* meal (D3) and fish fed 0% inclusion of toasted *Delonix regia* meal (D1) were not significantly different ($P>0.05$) from each other but were significantly higher than fish fed 20% inclusion of toasted *Delonix regia meal* (D4) ($P<0.05$). The WG and SGR of the tilapias fed experimental diets followed the same pattern as the final body weight. Survival of the fish fed experimental diets ranged from 95.22±1.05– 98.42±1.02%. Survival rate of D2 was significantly higher than the others ($P<0.05$) however there was no significant difference between D4, D3 and D1. The total feed intake of fish fed D2 and D1 were not significantly different and were higher than the other fish fed experimental diets, although D3 and D1 were not significantly different. Fish fed D4 showed the lowest survival rate, it was significantly lower than D1 ($P <0.05$) but not different from D3 ($P>0.05$). The FE and the PER showed the same trend as the survival rate while PR followed the same trend as the FI.

The proximate body composition of the tilapia fingerlings fed with the experimental diet is given in Table 4. Although there were no significant ($P>0.05$) difference in the moisture content of D1 and D2, D1 gave the highest moisture content of (74.1±1.2g). D3 was significantly ($P<0.05$) different from D4 with the lowest moisture content of (72.5±1.1g). The % crude protein of D2 was significantly higher compared to others, which was (16.1±1.5g), followed by D2. However, there was no significant (P>0.05) difference in the % crude protein of D3 and D4. D4 gave the lowest value of (15.3±1.2g). There was no significant (P>0.05) different in the ash composition of all the fish fed the experimental diet. D4 gave the highest lipid content of (6.7±0.4g), followed by D3. Though there was no significant (P>0.05) difference between D2 and D1, however D1 had the lowest lipid content of (5.2±0.7g).

DISCUSSION

Results showed that the values of water quality parameters throughout the experimental period did not vary with each other considerably and these water quality parameters values were within the acceptable range for freshwater fish culture especially tilapia (Balarin and Hatton, 1979).

El-Sayed (1999) reported that the suitability of various ingredient inclusions in fish feed in terms of growth performance vary greatly among fish species and experimental conditions. Figure1. depicts the body weight gain pattern of the fish fed the different experimental diets for 56 days under our experimental conditions. The best growth responses were obtained from fishes fed D2, which is 10% inclusion of toasted *D. regia* meal

Fapohunda (2012) stated that diets produced with poor quality raw materials and under adverse processing conditions have inferior nutritive value and have adverse effects on fish health. From the result obtained on the proximate composition of the major ingredients used in the formulation of the experimental diets (Table 1.) toasted *D. regia* meal was rich in protein and lipid content, although it was lower than the values reported by Grant et al 1991, Abdullahi et al 2004 and Adewuyi *et al* (2010*)* but were higher than the value reported by Alemede *et al* (2010). The lipid content was similar to the value reported by Abdullahi 2004. The Proximate composition values of the formulated experimental diets were similar as shown in table 2, hence the differences witnessed in the growth performance and nutrient utilization of the fishes fed the experimental diets may be attributed to the inclusion level of the toasted *D. regia* meal.

All the fish in this study showed normal growth and the inclusion of toasted *D. regia* did not have any adverse effect on their morphology and did not compromise their growth, survival, feed intake, and nutrient utilization. For all the four experimental diets evaluated, the growth performance showed progressive increase; implying that the growth performance of the fish fed the experimental diets was influenced by the level of inclusion of toasted *D. regia* meal. This was evident in their WG, SGR, FE, FI and nutrient utilization. Survival rate of the fish fed experimental diet for 56 days in this study was between 96-98%. The high survival rate could be attributed to non-toxicity of TDSM in the current study. Toasting method employed in processing the *D. regia* meal in this study proved very effective according to the result obtained as shown in Table 3. This may be due to improved digestibility of Protein, polysaccharides and metabolizable energy in addition to inactivation of anti nutritive factors, Fagbenro and Davis, (2003). The inactivation of anti nutritive factors would certainly have increase protein digestion and palatability. The results obtained showed that fish fed D2 (10% toasted *D. regia* inclusion) performed better than the control diet (0% toasted *D. regia* inclusion) and Fish fed D3 (15% toasted *D. regia* inclusion) compared favorably with D1 hence they were not significantly different from each other.

In the context of this study the fishes fed the experimental diets accepted and ingested actively all the experimental diets however there level of acceptability differs with the inclusion level of toasted *D. regia* as showed in Table 3. Growth parameters served as indicators of fish's ability to utilize and retain nutrients in a given diet (Bake *et al* 2009). From growth performance of the Nile tilapias fingerlings fed experimental diets in this study, Diet 2 recorded the best growth performance and nutrient utilization (Table 3 and figure 1). The Weight gain (WG) of young fish is usually a reliable indicator of nutritional adequacy of the diet (Cho and Watanabe, 1988). Diet 2 that is 10% inclusion of toasted *D.regia* gave the highest final body weight, % weight gain, survival rate, specific growth rate, total feed intake, feed efficiency, protein efficiency ratio and protein retention while there was no significant difference between fishes fed D1 and D3. This clearly showed that the fishes fed toasted *D.regia* were able to utilize the nutrients from the diet. Diet 4 gave the lowest values in the group. Tilapia readily accepted and exhibited good survival when fed plant materials. The reduction in the growth performance and nutrient utilization observed in the tilapia fed D4 is most likely due to the increased inclusion level of toasted *D. regia* meal which may have affected the palatability of the diet hence the lower feed intake. This is in agreement with the work of (Fagbenro 1999, Francis *et al* 2001, Siddhuraju and Becker and Bake *et al.*, 2012). They suggested that when alternative protein sources especially plant protein sources are high in fish diet, palatability and attractiveness of the diets may be negatively affected. In general it may reduce feed intake due to the unpalatability and hence, lead to low growth performance and feed utilization.

The fishes fed the experimental diets effectively utilized the diets as evident in their final body composition (Table 4).The body composition of all the fishes fed the experimental diet were higher than the initial body composition in all the parameters measured. The moisture of the fishes fed the experimental diets decreases with an increase in the inclusion level of toasted *D.regia* meal while the lipid increased with an increase in the inclusion level of toasted *D.regia* meal .This result is in line with those reported by Mohsen and Lovell (1990); Sorrano *et al.* (1992); Yildirim *et al.* (2003); Luo *et al.* (2005) and Bake *et al.*, (2012). They reported that fat content is closely related to the body moisture content. This study Showed that an increase in the inclusion level of toasted *D.regia* influenced the accumulation of lipid content in the fishes fed the experimental diets. This result also agreed with Hanley (1991) who reported that tilapia has the ability to store significant quantities of lipid in carcass and may not utilize them as energy source to improve its growth performance. The fish fed diet 2 gave the highest crude protein while diet 4 gave the lowest although, the percentage crude protein of D1, D3 and D4 were not significantly ($P>0.05$) different from each other. Likewise the ash content were not significantly ($P>0.05$) different in all the diets.

From the result obtained in this study, it is concluded that Nile tilapia fingerlings (*Oreochromis niloticus)* can make use of toasted flamboyant seed meal (*Delonix regia)* at an inclusion level up to 15 % in their diets to give excellent performance in growth, nutrient utilization and body composition without any adverse effect on there body. However and increase beyond this would lead to depression in their growth response. For effective utilization of toasted *D. regia* meal there is need to evaluate the environmental impact of this toasted *Delonix regia* seed meal base diet in a long-term pond culture system. Therefore further research on the aspect of digestion and mechanism of action of anti-nutritional factors is needed

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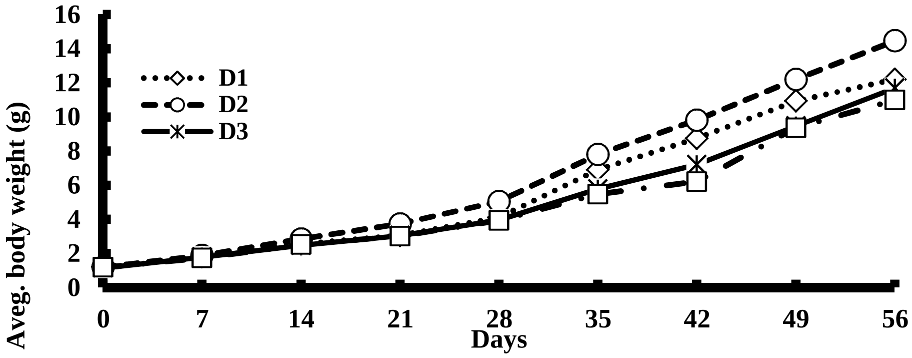

Fig. 1 Growth of *Oreochromis niloticus* Juveniles fed the experimental diets for 56 days

Table 1. Proximate composition of the major Ingredients used for the experimental diets

Ingredients	Fish meal	Soybean meal	Toasted *D. regia* meal	Maize meal	Millet meal
Moisture (%) d.b[*1]	6.21	4.57	3.24	5.09	5.63
Crude protein (%) d.b	61.25	48.07	26.20	9.32	8.98
Lipid (%) d.b[*1]	10.70	7.04	9.82	4.20	3.56
Ash (%) d.b[*1]	6.69	4.35	6.48	3.84	3.60

[*1] Dry basis

Table 2. Formulation and proximate composition of the experimental diets for *Oreochromis niloticus* fingerlings

Ingredients	D1	D2	D3	D4
Fishmeal	394.00	350.00	330.00	308.00
Soybean meal	200.00	200.00	200.00	200.00
Toasted *delonix regia* meal	0.00	100.00	150.00	200.00
Maize meal	70.00	70.00	70.00	70.00
Millet	70.00	70.00	70.00	70.00
Starch	93.00	55.00	55.00	55.00
Vitamin premix	25.00	25.00	25.00	25.00
Mineral	25.00	25.00	25.00	25.00
Soybean oil	30.00	30.00	30.00	25.00
Cellulose	93.00	75.00	45.00	22.00
Total	1000.00	1000.00	1000.00	1000.00
Moisture	5.44	5.27	5.18	5.32
Crude protein	34.51	34.96	33.93	33.93
Lipid	9.35	9.40	9.25	9.44
Ash	10.62	10.51	10.14	9.94

Table 3. Growth performance and nutrient utilization of *O. niloticus* fed experimental diets for 56 days

Diet code	Body weight (g)		Weight gain (%)	Survival rate (%)	Specific growth rate (%)	Total feed intake (g)	Feed efficiency	Protein efficiency ratio	Protein retention (%)
	Initial	Final							
D1	1.21±0.04	12.32±0.01^{b}	1132.00±1.00^{b}	96.50±1.14^{b}	2.76±0.01^{b}	11.56±0.10^{ab}	0.96±0.01^{b}	2.79±0.03^{b}	43.71±0.44^{b}
D2	1.20±0.07	14.26±0.43^{a}	1326.00±43.59^{a}	98.42±1.02^{a}	2.95±0.04^{a}	12.53±0.10^{a}	1.04±0.04^{a}	2.98±0.06^{a}	48.67±0.91^{a}
D3	1.15±0.06	11.29±0.54^{b}	1029.67±54.72^{b}	96.36±1.07^{b}	2.72±0.06^{b}	11.02±0.72^{bc}	0.92±0.01^{b}	2.72±0.03^{b}	42.25±0.51^{bc}
D4	1.18±0.02	9.89±0.64^{c}	889.33±64.33^{c}	95.22±1.05^{b}	2.53±0.08^{c}	9.57±0.84^{c}	0.91±0.01^{b}	2.69±0.04^{b}	41.66±0.69^{c}

Values in the same column with different superscript letters are significantly different ($p<0.05$) from each other.

Table 4 Proximate composition analyses of whole body O. niloticus (wet basis) fed experimental diets for 56 days

Component (%)	Initial	Final*[1]			
		D1	D2	D3	D4
Moisture	75.64	73.08±1.2^{a}	72.66±0.2^{ab}	71.56±0.3^{c}	71.45±1.1^{d}
Protein	14.94	15.72±1.1^{b}	16.12±1.5^{a}	15.39±1.1^{b}	15.32±1.2^{b}
Lipid	5.19	6.15±0.7^{c}	6.05± 0.5^{c}	7.18±0.5^{b}	7.65±0.4^{a}
Ash	4.63	5.17±0.2^{a}	5.17±0.3^{a}	5.3±0.1^{a}	5.42±0.3^{a}

*[1]Values in the same row with different superscript letters are significantly different ($p<0.05$) from each other ($n=3$).