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Evaluation and Nutrient Quality of Toasted Flamboyant Seed (*Delonix regia*) Meal in the Diet of *Clarias gariepinus* Fingerlings

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Authors' contributions

This work was carried out in collaboration between all authors. Author GGB designed the study, wrote the protocol and wrote the first draft of the manuscript in conjunction with author IY who also managed the literature searches, analyses of the study performed the structural equation modelling. Author SOES wrote the discussion and conclusion of this work. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

56 days feeding trial was conducted to evaluate the effect of toasted *Delonix regia* seed meal (TDRSM) in the diet of *Clarias gariepinus* fingerlings with respect to their growth performance, feed efficiency, nutrient utilization, and body composition. Four iso-nitrogenous and iso-lipidic experimental diets were formulated to contain 0%, 10%, 20%, and 30% of toasted *Delonix regia* meal and were designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), and D4 (30% inclusion) in a completely randomized design. Fifteen net hapa (0.5x0.5x1 m) were suspended in two outdoor concrete ponds with the aid of kuralon twine tied to plastic poles, the concrete ponds were filled to 5/6 of its volume (40 m³) with filtered and dechlorinated tap water. Each treatment had three replicates with 20 fish were accommodated in each hapa (mean initial



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body weight (1.02±0.01 g) per fish. Water temperature and other water quality parameters were monitored daily. The result of the study showed that there was no significant differences (*P*>0.05) in the survival rate, However D3 gave the best value in all the growth parameters values measured and was significantly different from others while D4 gave the lowest value but was not significantly different from D1. Except for D3 which was significantly higher in all the nutrient utilization values measured however was no significant difference between D1, D2 and D4. Proximate composition results revealed that increase in inclusion of TDRM led to an increase in the carcass lipid content and a decrease in moisture contents of the fish fed experimental diet. This result indicated, that inclusion of toasted *Delonix regia* meal at 20% in the diet of *Clarias gariepinus* fingerlings improves the nutritive value for fish, hence can serve as alternative ingredients in the fish feed production.

Keywords: Clarias gariepinus; growth performance; Delonix regia; toasted seed meal.

1. INTRODUCTION

With aquaculture gaining global attention on recent time, and fish being the cheapest source of animal protein however, due to the decline in the wild catches the industrialized fishing sector has become unpredictable and unsustainable. Hence aquaculture production intensifications is paramount importance especially of the developing nations of the world [1]. One of the major concerns in aquaculture production is the high cost of the feed and heavy reliance on fish meal and fish oil as the primary protein and energy source in aqua feed production. Fish meal is very palatable and has exceptional nutrition value including an excellent balance of essential amino acid and essential fatty acid. which closely meet the requirement of most farm fish, and also provides an excellent source of digestive energy and vitamin [1]. The high cost, scarcity and insufficient supply of some of these sources plant conventional protein has necessitate the need for a search for alternative unconventional plants protein ingredients [2]. Obun, [3] suggested that the alternative plant protein source to replace conventional ones may have comparative nutritive value or preferably be cheaper than the conventional protein sources i.e. feed ingredients that are rarely consumed by human and less competed for by the livestock sector, hence locally available and cheap feedstuff are of paramount interest to aquafeed producers and fish farmers especially those from the sub-Sahara.

Several feed ingredients have been investigated in an attempt to find alternatives for soybeans in the diets of fishes. These include plant protein sources such as cottonseed meal, groundnut meal, sunflower, rapeseed, sesame seed, copra, macadamia and palm kernel along with aquatic plants such as *Azolla pinnata*, duckweed (Lemnaceae) and single-cell proteins [4]. These feeds are not only considerably cheaper than soybeans but also enjoy high availability and accessibility in certain regions of the world especially in Nigeria.

To attain a more economically sustainable and viable production, research interest has be redirected towards the evaluation and use of unconventional plants protein sources [5,6,7].

Delonix regia is a wild plant otherwise called flame of the forest, it is an ornamental, leguminous plant, which produces tones of pods containing seed in the fruiting season [8]. These seeds are left unutilized since they are neither consumed by any animal nor utilized for any other medicinal purpose. It produces 25-40 cm long pods containing seeds which laboratory analysis has reported to contain crude protein of 36.92%, crude lipid: 4.17%, and crude fiber: 11.39% [9]. Catfish Clarias gariepinus belongs to family of Claridae, they are the most cultured freshwater fish in Nigeria. They are characterized by their ability to grow on a wide range of artificial and natural feeds, they grow fast and have high potential hardness and tolerance to dissolved oxygen and other aquaculture routine [10]. Hence emphasis has been on the increase on how to improve much productivity of catfish. This study was conducted to evaluate the suitability of toasted Delonix regia seed meal (TFSM) in the diet of Clarias gariepinus fingerlings through their growth performance, nutrient utilization and haematological indices.

2. MATERIALS AND METHODS

2.1 Ingredients and Diet Formulation

2.1.1 Soybean meal

Soybean was obtained from the Kure's new modern market and was toasted using a frying

pan and allow to cool before milling with the aid of a grinding machine. Toasting was done to remove the anti-nutritional factor present in the seed.

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 tables shown in Table 1.

2.1.2 Flamboyant seed meal

Matured and dried Flamboyant seedpods were collected from ornamental garden of the centre for preliminary and extra moral studies (CPES) of Federal University of Technology Minna. The seed were collected by manually opening the pods. The viable seeds were sorted out by soaking the seed inside water. The viable seed were sun dried and seeds were toasted within the temperature range of 60°C to 80°C to reduce the effect of anti-nutritional factor using frying pan. After toasting, the seed were allow to cool and later grounded to powder.

2.1.3 Fishmeal

The fish meal used for these experiments was obtained from Mosegoaler farms Minna Niger state. The crude protein and lipid were 65.34% and 11.36%.

2.1.4 Experimental diets

Based on the nutritional requirements of *Clarias gariepinus* fingerlings [11], four isonitrogenous and isolipid diets were formulated at 40 % protein and 9.5 % lipids, containing 10-30% toasted *Delonix regia* seed meal at different levels of inclusion (Table 3).

2.1.4.1 Experimental system and fish

The experimental fish, pure- bred *C. gariepinus* fingerlings, with an initial mean weight of (1.01 - 1.02 g) were purchased from Tagwai fish hatchery of Ministry of Livestock and Fisheries development Minna, Niger state. The fish were transferred in a well-oxygenated water plastic container from the hatchery to the Department of Water Resources, Aquaculture and Fisheries Technology experimental fish farm, Federal University of Technology, Bosso campus, Minna where the feeding trial was conducted. Upon arrival they were acclimatized in a transitional tank in the farm for four days and were fed

commercial feed (coppense feed) at 40% crude protein once a day before the experiment commenced. The fish were subsequently fed with 40% iso-nitrogenous diet and 9.5% lipid, containing different inclusion level of toasted toasted Delonix regia seed meal, designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion), D4 and (30% inclusion) for 56 days. Fifteen net hapa (0.5x0.5x1m) were suspended in two outdoor concrete tanks (8mx5mx1.5m) with the aid of kuralon twine tied to plastic poles. The concrete tanks were filled to 5/6 of its volume (40 m³) with filtered and dechlorinated tap water. Twenty (20) fish were accommodated in each hapa and each treatment was randomly allocated to three hapas. Photoperiod depends on the natural light, and water temperature was monitored daily. The water quality parameters in the system were monitored weekly, the temperature ranged between 24°C-29°C 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. Two replicate of each treatment using 20 fish per hapa were reared on each of the four diets. The feed was manually administered and the fish were fed three times daily at 5% of body weight at 09:00 am, 12:00 pm and 16:00 pm. Feeding rate was subsequently adjusted according to their growth rates per hapa. The uneaten and faecal matters were siphoned out of the hapa every morning before feeding, and 45 minutes after the fish have been fed. The fish were denied feed 24 h prior to sampling. Five fish were randomly sampled on weekly basis, and weights were measured using a digital electronic weighing balance (CITIZEN MP-300) model.

2.2 Biochemical Analysis

About 10 g initial sample and 15 g of final samples from each hapa were pooled separately and then homogenized using laboratory mortar and pestle. The major ingredient used for the diet; the formulated diet and the fish body samples were subjected to chemical analysis. The proximate composition analysis was determined according to AOAC procedures [12]. Moisture content was determined by drying samples at 105±2℃ 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 nhexane 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 [12]. Anti-nutritional factors of the seeds; tannins and trypsin inhibitor activity (TIA) were analyzed by modifying the procedures of [13]. Phytic acid was determined by the method of Latta and Eskin [14].

2.2.1 Blood collection and haematological analysis

Blood samples were collected in triplicate following the procedure of Klontz and Smith [15,16] and Wedemeyer and Yasutake, and subsequently taken to the Laboratory of Department of Biochemistry Federal University of Technology Minna for haematological analysis. The direct measurement of erythrocyte values (Packed cell volume PCV, Haemoglobin Hb, and Red blood cell RBC) and absolute erythrocyte indices (MCH, MCV and MCHC) were calculated. The white blood cell and differential counts (neutrophils and lymphocytes) were analyzed as described by Dacie and Lewis [17].

2.2.1.1 Evaluation of blood parameters

MCV=PCV/ Erythrocytes count ×10 MCH=Haemoglobin / Erythrocytes count ×10 MCHC= Haemoglobin /PCV ×100

2.2.2 Evaluation of growth parameters

Growth performance and diet nutrient utilization were analyzed in terms of weight gain (WG), feed efficiency (FE), specific growth rate (SGR), feed intake (FI) protein efficiency ratio (PER) and apparent protein digestibility. The following formulas were used:

WG (%) = (final weight (g) - initial weight (g)) / initial weight (g) \times 100

FE (%) = weight gained (g) / feed fed (g) ×100

SGR (%) = ((In final weight (g) - In initial weight (g)) / feeding period (day) $\times 100$

FI (mg/fish/day) = dry feed (mg) fed / number of fish / feeding period (day)

PER = wet body weight gain (g) / protein intake (g)

2.3 Statistical Analyses

Data were analyzed using one-way analysis of variance (ANOVA) using Statistica 8.0 (Stat-Soft,

Inc., Oklahoma, USA). Differences between treatments were compared by Tukey's test. Level of significance was tested at *P*<0.05.

3. RESULTS AND DISCUSSION

This study investigates the possibility of utilizing toasted Delonix regia meal in the diet of Clarias gariepinus fingerlings. Table 1 shows the proximate composition of the major ingredients used in formulating the experimental diets. Fish meal has the highest crude protein and lipid content (65.34% and 11.36%) followed by soybean meal (43.63% and 7.00%), while the crude protein and lipid content of both the raw and toasted Delonix regia meal was (22.45-29.58% and 5.68-9.39%) respectively. The value of the D. regia reported in this experiment was higher than the value reported earlier by Balogun et al. [18] but lower than the value reported by Bake et al. [19] the differences might be attributed to differences in environmental conditions and the processing technique used. The increase in the protein and lipid content of the TFSM may be attributed to the processing technique used, this is in line with [20,21,22,23] that heating and toasted of plant protein base ingredients boost their proximate composition especially protein and lipid. Table 2 showed the anti-nutritional factor composition of both the untreated raw D. regia (RFSM) and the treated toasted D. regia (TFSM). All the anti-nutritive factors parameters measured were lower in the treated TFSM ingredient as compared to RFSM. The lower values of the anti- nutritive factors may be as a result of the toasting of the flamboyant seed, this agrees with the findings of [7,21,22] that heat and toasting processing technique usually reduced the level of anti-nutritive factors in plant protein based ingredients. It could be seen from Table 3 that the diets used in the present study were isonitrogenous and all experimental diets were similar in terms of their chemical and proximate composition. The composition of the diets indicates that, increase in the inclusion level of TFSM in D2, D3, and D4 did not have any negative affect on their physical and chemical properties. This further affirms that Delonix regia seed as a fish feed ingredient may not be a starchy material. The food quality of a diet is directly proportional to its ability to support growth, and its dietary content is of paramount importance. One of the limiting growth factors in fish is protein, and it is well known fact that it is essential for building new tissues [24]. The good growth of C. gariepinus fed the tested diets in the present study indicated that the protein values of these diets were appropriate despite being lower than the 40% formulated on paper.

The growth performances of *C. gariepinus* fingerlings in terms of WG, SGR, FE, FI PER and PR are shown in Table 4. Among the formulated experimental diets, *C. gariepinus* fingerlings fed on D3 (20% inclusion of TFSM) gave the best and the highest significant (P<0.05) value in all the growth parameters measured while D4 (30% inclusion of TFSM) had the lowest WG, SGR, and FI, although there was no significant (P<0.05) differences between fish fed D4 and D1 based diets. Fish fed D2 had a higher significant (P<0.05) growth performance value than those fed D1 and D4.

In the nutrient utilization parameters measured (FE, PER and PR) fish fed D3 had the highest value and was significantly different from other fish fed other experimental diets. However there was no significant difference between D1 and D2 and D4.

Fish mortality was low and relatively uniforms in all the treatments, furthermore all the experimental fish remaining in the hapas were morphologically normal at the end of the feeding trial. In this present study the water temperature which ranges between 26±0.5°C-29±0.7°C and the dissolved oxygen 6.5 to 8.4 mg/L were within the acceptable range for catfish culture [25].

 Table 1. Proximate composition of the major ingredients used in the formulation of the experimental diet for *C. gariepinus* fingerlings

Ingredients	Fishmeal	Soybean meal	Maize meal	Millet meal	RFSM	TFSM
Proximate composition						
Moisture (%)	5.79	2.09	4.66	3.22	4.02	3.78
Crude protien (% d.b. ^{*1})	65.34	43.63	9.32	12.86	22.45	29.58
Crude lipid (% d.b. ^{*1})	11.36	7.00	4.20	4.36	5.68	9.39
Ash (% d.b. ^{*1})	14.34	8.15	3.22	2.33	6.35	4.14

Anti-nutritive factors	RFSM	TFSM	(%) decrease of anti-nutritive factors after fermentation
Phytic acid (mg/100 g)	540.07	141.33	73.83
Cyanide (mg/100 g)	19.45	7.15	63.24
TIA (mg/100 g)	38.92	10.21	73.77
Tannin (g/kg)	22.74	8.82	59.43
Total oxalate	45.46	10.15	77.67

Table 2. Effect of toasting treatment on anti-nutritional factors in Delonix regia seed meal

Table 3. Formulation of the experimental diet and proximate composition of the experimental diet for *C. gariepinus* fingerlings (g/kg)

Ingredients	D1	D2	D3	D4
FM	529.30	483.80	438.50	393.00
SBM	100.00	100.00	100.00	100.00
TDRM	0.00	100.00	200.00	300.00
MM	50.00	50.00	50.00	50.00
Millet	50.00	50.00	50.00	50.00
starch	55.00	55.00	55.00	55.00
Vitamin premix	15.00	15.00	15.00	15.00
SBO	24.30	20.10	15.90	11.70
Cellulose	161.40	111.10	60.60	10.30
Mineral premix	15.00	15.00	15.00	15.00
Total	1000.00	1000.00	1000.00	1000.00
Moisture (%)	4.18	4.29	4.15	4.31
Crude protien (% d.b. ^{*1})	37.51	37.98	37.46	37.22
Crude lipid (% d.b. ^{*1})	8.75	8.82	8.66	8.92
Ash (% d.b. ^{*1})	12.52	12.63	12.78	12.95

Diet	Body weig	ıht (g)	Weight gain	Survival	Specific growth	Total feed	Feed	Protein	Protein
code	Initial	Final	(%)	rate (%)	rate (%)	intake (g)	efficiency	efficiency ratio	retention (%)
D1	1.02±0.04	13.25±0.05 [°]	1199.02±14.32 ^c	98.80±1.14 ^a	4.58±0.01 [°]	16.95±0.31 ^b	0.72±0.01 ^b	1.92±0.05 ^b	34.50±0.24 ^b
D2	1.01±0.06	14.03±0.28 ^b	1289.11±27.51 ^b	98.96±1.07 ^a	4.70±0.04 ^b	17.78±0.17 ^a	0.73±0.01 ^b	1.95±0.02 ^b	34.59±0.38 ^b
D3	1.02±0.06	15.73±0.03 ^a	1442.16±37.25 ^ª	98.97±1.02 ^a	4.88±0.02 ^a	17.58±0.12 ^ª	0.84±0.04 ^a	2.20±0.08 ^a	40.18±0.75 [°]
D4	1.02±0.02	13.18±0.03 ^c	1191.83±32.22 ^c	98.82±1.05 ^ª	4.57±0.06 [°]	16.75±0.12 ^b	0.73±0.03 ^b	1.95±0.03 ^b	34.42±0.25 ^b

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

Table 5. Proximate composition analyses of whole body C. gariepinus (wet basis) fed experimental diets for 56 days

Component (%)	Initial	Final ¹				
		D1	D2	D3	D4	
Moisture	77.82	75.02±1.4 ^a	73.76±1.3 ^b	72.45±1.4 ^c	71.82±1.4 ^d	
Protein	13.45	17.59±1.7 ^b	17.39±1.7 ^a	17.93±1.2 ^c	17.32±1.5 [°]	
Lipid	3.87	$4.47 \pm 0.6^{\circ}$	$4.72 \pm 0.6^{\circ}$	5.98±0.2 ^b	6.97±0.7 ^a	
Ash	2.96	3.21±0.4 ^a	3.22±0.4 ^a	3.25 ± 0.4^{a}	3.28±0.6 ^a	
	$\frac{1}{2}$					

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

Table 6. Haematological parameters of C. gariepinus fingerling fed experimental diets for 56 days

Blood parameters	Initial	Final ¹¹				
		D1	D2	D3	D4	
PCV (%)	20.62	28.34±1.27 ^a	28.42±1.34 ^a	28.68±1.36 ^a	26.55±1.47 ^b	
WBC (10^3mm^{-3})	7.18	7.29±0.24 ^b	7.37±0.28 ^b	7.41±0.52 ^b	7.67±0.32 ^a	
RBC (10^3mm^{-3})	2.56	3.28±0.35 ^a	3.24±0.26 ^a	3.18±0.42 ^a	2.95±0.38 ^b	
Hb (g/100 ml)	6.19	8.61±0.67 ^a	8.87±0.24 ^a	8.96±0.43 ^a	8.02±0.52 ^b	
LYMPH (100)	60.15	61.40±4.47 ^b	61.66±3.33 ^b	62.38±2.54 ^a	61.45±3.27 ^b	
MCHC (%)	30.02	30.40±1.55 ^a	31.21±1.23 ^a	31.24±1.61 ^a	30.21±1.28 ^a	
MCH (pg)	24.18	26.25±1.88 ^b	27.38±1.76 ^b	28.19±1.42 ^b	27.19±1.65 ^ª	
MCV (fl)	80.55	86.39±5.36 ^a	87.72±3.48 ^a	90.25±6.32 ^a	90.00±3.28 ^a	

PCV, Packed cell volume; WBC, white blood cell; RBC, red blood cell; Hb, haemoglobin; LYMPH, lymphocyte; MCHC, mean corpuscular haemoglobin concentration; MCH, mean corpuscular haemoglobin; MCV, mean corpuscular volume.

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

There was variations in the proximate composition analysis of whole body Clarias fingerlings gariepinus (wet basis) fed experimental diets for 56 days, as showed in (Table 5 above). The results of the body composition revealed that except for moisture catfish fed toasted Delonix regia meal display a marginal increase in carcass protein, lipid, and ash, at the end of the experiment and were significantly higher than the initial body composition at the start of the experiment. There were no significant differences in the carcass ash contents among all the treatments fed experimental diets, fish fed D3 had the highest crude protein content value and was significantly higher than the other fish fed other experimental diets (P<0.05), fish fed D1was significantly higher (P<0.05) than those fish fed D2 and D4 however D2 and D4 do not show any significant different (P>0.05) from each other. Fish fed D4 had the highest significant lipid carcass composition (P<0.05) while D1 had the lowest value but was not significant different from fish fed D2, fish fed D1 show the highest moisture among experimental diets although no significantly different from D2 (P<0.05) but were significantly different from D3 and D4.

In the present study, the inclusion of TFSM significantly influenced the proximate composition of fish muscle tissue. Increased crude fat deposition was observed in response to increase in the levels of toasted flamboyant seed inclusion, in this circumstance, the lipid was inversely proportional to moisture. This result then to agree with the findings of [26,27,28,7,29]. When plant base protein source are included in fish diets there is tendency for increase in the carcass lipid.

Table 6 (above), revealed the haematological parameters of the experimental fish before and after the feeding trial. Fish fed the experimental diet had a higher value in all the Haematological parameters measured as at the end of the experiment as compared to the initial before the feeding trial. The fish fed D3 had the highest Packed Cell Volume ,while fish fed D4 had the lowest PCV value and was significantly (p<0.05) different from those fed the other experimental diets. However there was no significant difference between fish fed D1, D2 and D3 (P > 0.05). WBC values showed that fish fed D4 had the highest value and was significantly different from others (P<0.05), although D1 had the lowest WBC value it was not significantly

different from D2 and D3 (P > 0.05). The RBC and Hb followed the same pattern as PCV. LYMPH showed that D3 was higher and significantly different from other fish fed experimental diets, however there was no significant differences between D1, D2 and D4. While MCH followed the same pattern as WBC, there was no significant difference in MCHC and MCV among all fish fed the experimental diets (P>0.05).

The results of the Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell (RBC), and White blood Cell (WBC) in this present study were within the acceptable range of a healthy C. gariepinus fingerlings [30,31], indicating that TFSM is not toxic to Clarias gariepinus fingerlings, and are safe to feed up to 30% level of inclusion. The values obtain in this study was higher than those reported hv [30,31,32,33,34,35]. The increase in all of the hematological parameters measured in all the fish fed the experimental diets is an indication that there was no blood lost in fish fed those diets when compared with what was reported by Sotulo and Faturori [33], when Leucaena leucocephala was fed to Clarias gariepinus fingerlings.

In summary the growth performance of C. gariepinus fed toasted flamboyant seed meal diets as presented in Table 4 shows that there was no feed rejection during the experiment, the diets were accepted by the fish and they ingested the feeds actively, although the acceptability differs among the treatment as could be seen in their total feed intake. When plant materials are used in fish diets, one common problem is usually the acceptability of the diet by the fish which has to do with the level of inclusion and palatability of the diet [21]. Proper processing of ingredients has been reported to have effects on the texture, palatability, and the level plant material incorporated, hence can affect the acceptability and level of feed intake [20,21,22,23]. In this present study the high FI obtained in this feeding trial may also be ascribed to the toasting processing technique employed in processing the flamboyant seed. This may have reduced the effect of anti-nutritive factors and other secondary compound activities in the seed which tend to have enhance the palatability and boosted the feed intake as evident in the high feed intake of all the fish fed the experimental diets.

4. CONCLUSION

In conclusion, the results of this study demonstrate that 10–30% of TFSM can be included in the diet of *C. gariepinus* fingerlings without any adverse effect on their growth performance and blood system. Further research need to be carry out on the amino acid and essential fatty acid profile of the toasted flamboyant seed on a long term study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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