

## Nutrient Utilization and Haematological Indices of *Clarias gariepinus* Fingerlings Fed Varying Inclusion Levels of Fermented Flamboyant (*Delonix regia*) Seed Meal in Concrete Tanks

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### ABSTRACT

This study investigates the nutrient utilization and hematological indices of African cat fish *Clarias gariepinus* fingerlings fed fermented flamboyant (*Delonix regia*) seed meal (FFSM). Fish with mean initial weight of  $1.1 \pm 0.1$  g were fed four isonitrogenous and isolipidic diets formulated at 40% crude protein and 9.5% lipid containing different levels of inclusion of FFSM and designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion) D4 (30% inclusion) for 56 days. Twenty fish per hapa were accommodated in twelve net hapa ( $0.5 \times 0.5 \times 1$  m) suspended in two outdoor concrete ponds ( $8 \times 5 \times 1.5$  m) with the aid of kuralon twine tied to plastic poles. The concrete ponds were filled to 5/6 of its volume ( $40 \text{ m}^3$ ) with filtered and dechlorinated tap water. The fish were fed at 5% body weight three times daily. The results showed that fish fed D4 had the highest significant value in the Total Feed Intake (TFI) although not significantly ( $p > 0.05$ ) different from D1 and D2. Fish fed D3 had a higher feed utilization, hence, feed efficiency, protein efficiency ratio and protein retention were significantly ( $p < 0.05$ ) higher than those fed D1, D2 and D4 fed fish. There was no significant difference in the hematological indices among all the fish fed the experimental diets. In conclusion, inclusion of FFSM up to 30% in the diet of *Clarias gariepinus* is suitable and have no negative effect on the nutrient utilization and hematological indices of the fish fed experimental diets. Hence FFSM can be a potential ingredient for aqua feed industry.

**Key words:** *Clarias gariepinus*, growth performance, flamboyant seed meal, hapas

## INTRODUCTION

Today, over 49% of the fish consumed is produced by aquaculture. This industry has been growing at an average rate of 8.8% per year since 1950 to keep pace with the global demand for fish products (FAO, 2006). To sustain this unprecedented expansion in aquaculture sub sector, research and development of fish farming techniques are required, in order to obtain the most efficient, safe and cost effective methods for producing aquacultural product.

One of the main concerns for rearing finfish in an intensive 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 (Tacon 2008; Bake *et al.* 2009). The protein constituent represents the highest cost in feed production and ultimately fish farming because of the relatively high percentage of fish meal that has to be incorporated into the diets there by making fish feed very expensive which in turn affect the aquaculture industry adversely.

Therefore, the future of large scale aquaculture will depend largely on the effective use of alternative proteins inclusion in practical diets for fish. Plant proteins are more available, relatively cheaper but contain less protein than fishmeal. The search for alternatives to fishmeal has largely been focused on conventional sources such as oil seed, cakes and meals due to their relatively higher protein content. These conventional plant protein sources include soybean, groundnut, sunflower, rape seed and cottonseed cake.

Despite their usefulness, these ingredients are scarce and relatively expensive due to high demand for livestock production and other industrial use. Moreover, their cultivation generally requires high use of inputs and energy subsidies (Francis *et al.*, 2002). This makes them unaffordable, unsustainable and sometimes even conflicts with food security interests, particularly among resource-poor farmer.

Flamboyant tree (*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 compares 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. Sorensen *et al.* (2009) and Akinmutimi (2001), reported that there are anti-nutritional factors associated with plant proteins hence, plant-derived ingredients still have limited application in aqua feed production, because of the complexity of nutrients and anti-nutritional compounds commonly present in typical plant-derived ingredients, both of which reduce nutrient availability to fish (Fagbenro and Davies 2003). Development of plant-derived ingredients that are very digestible and with less negative factors affecting digestion and metabolism in fish is of paramount importance to aqua feed researchers and producers.

Plant-derived ingredients can better be put to use when properly processed, in aquaculture there are various processing techniques that can be applied to allow better and proper plant-derived ingredients utilization.

Fermentation has long been used to prepare healthy foods for humans and livestock (Kim *et al.*, 1999; Lee, 2000). Fermentation is a unique process which usually improve the nutritional value of feed ingredients. Fermentation process is critical to denature and destroy or reduce anti-nutritional factors but not damage nutrient protein fractions. Furthermore, fermentation process has the ability to remove a substantial portion of the glycinin and  $\beta$ -conglycinin (Helm *et al.*, 2000; Rickert *et al.*, 2004; Deak *et al.*, 2006), which are potential antigenic and allergenic compounds (Holzhauser *et al.*, 2009). This fermentation process provides a promising future for plant-derived ingredients for sustainable aquaculture.

A haematological study in fish nutrition is gaining more attention of fisheries researchers. This is because of its importance in monitoring the physiological condition and health status of the cultured fish (Hrubec *et al.*, 2000) which

serves mainly for diagnostic purpose hence can be used to appraise the suitability of feeds and feed mixture pellets, to examine the effect of stress situation and so on. (Svobodova *et al.*, 1991. Changes in haematology of fish in response to stressing agents are indicators of the distress stage of fish, giving vital information to control any unfavourable condition that may affect the health status of the fish (Bello-Olusoji *et al.*, 2006). Furthermore, the knowledge of haematological characteristics of the fish is important in toxicological studies and its implication on final consumers which is man. In culture fisheries, these studies are usually associated with the feed input. The red Blood Cells Count (RBC), haematocrit (PCV) and haemoglobin (Hb) concentration vary with diet and strain as well as temperature, season of the year and nutritional status of the fish (Barnhart, 1969). Although *Delonix regia* seeds are in abundance not much work has been done on its utilization as an ingredient in the diet of *C. gariepinus* fingerlings and its subsequent impact on the haematological indices of the fish. It is in the light of this that this study was carried out with the main objective to investigate and evaluate the nutrient utilization and haematological indices of *C. gariepinus* fingerlings fed varying inclusion levels of fermented flamboyant seed meal (*Delonix regia*) in a concrete tank.

## MATERIALS AND METHODS

### Ingredients and diet formulation

**Soybean meal (SMB):** Raw soybean was purchased from the Bosso Market Minna (Niger State). The soybean was processed by toasting in frying pan at 80°C for 60 min until the colour changed to golden brown and allowed to cool before milling in a disc attrition machine. Crude protein and lipid contents of SMB were 43.63 and 7.00%, respectively as shown in Table 1.

**Fishmeal (FM):** The fishmeal used in this experiment was obtained from the Musgola Fish Farm, along Bosso Estate Road Minna Niger State, Nigeria. The crude protein and lipid content of fishmeal were 65.34 and 11.36%, respectively as shown in Table 1.

**Fermented deloxi regia seed meal (FFSM):** Deloxi regia seed pods were collected manually during the dry season from the botanical garden of Centre for Preliminary and Extra Moral Studies (CPES) Federal University of Technology Minna Niger State. It was manually crushed to get the seed. The fermentation of the seeds was carried out by mixing the *D. regia* seed with water in the ratio 2:1 (1 part of *D. regia* seed to 2 parts of water); 0.25 mL of cultured *Aspergillus niger* which was collected from the Department of Microbiology Laboratory of Federal University of Technology Minna, was pipette and mixed with the water. The mixture was packed in a plastic container, firmly sealed with cotton wool and kept at ambient temperature of 25°C. The sample was fermented for five days. The fermented sample was then washed and spread on a polythene sheet in a room and dried for 6 days up to about 90% of the dry matter. The seed was grinded into powder using hammer mill. Crude protein and lipid contents of FFSM were 36.42 and 8.14%, while RFSM were 22.15 and 6.68%, respectively as shown in Table 1.

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 formulation and proximate composition table is shown in Table 2.

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	FFSM
<b>Proximate composition</b>						
Moisture (%)	5.79	2.09	4.66	3.22	4.02	4.78
Crude protien (% d.b.*1)	65.34	43.63	9.32	12.86	22.15	36.42
Crude lipid (% d.b.*1)	11.36	7.00	4.20	4.36	6.68	8.14
Crude fiber (% d.b.*1)	0.65	5.05	3.43	2.62	8.56	4.72
Ash (% d.b.*1)	14.34	8.15	3.22	2.33	5.35	4.34

d.b\*1: Dry bases, RFSM: Raw flamboyant seed meal, FFSM: Fermented flamboyant seed meal

Table 2: Effect of fermenting treatment on anti-nutritional factors in *Delonix regia* seed meal

Anti nutritive factors	RFSM	FFSM	Decrease of anti-nutritive factors after fermentation (%)
Phytic acid (mg/100 g)	540.07	105.20	80.52
Cyanide (mg/100 g)	19.45	5.24	73.06
TIA (mg/100 g)	38.92	9.34	76.00
Tannin (g kg <sup>-1</sup> )	21.74	6.02	72.31
Total oxalate	45.46	7.65	83.17

RFSM: Raw flamboyant seed meal, FFSM: Fermented flamboyant seed meal

**Experimental diets:** Based on the nutritional requirements of *Clarias gariepinus* fingerlings (NRC 1993), four isonitrogenous and isolipidic diets were formulated at 40% protein and 9.5% lipids, containing 10-30% FFSM at different levels of inclusion.

**Experimental conditions and fish rearing:** The experimental fish, pure-bred *C. gariepinus* fingerlings, with an initial mean weight of (1.01-1.01 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, Minna Bosso Campus, 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 FFSM designated as D1 (0% inclusion), D2 (10% inclusion), D3 (20% inclusion) and D4 (30% inclusion) for 56 days. Fifteen net hapa (0.5×0.5×1 m) were suspended in two outdoor concrete tanks (8×5×1.5 m) with the aid of kuralon twine tied to plastic poles. The concrete tanks were filled to 5/6 of its volume (40 m<sup>3</sup>) with filtered and dechlorinated tap water, Twenty fish were accommodated in each hapa. Each treatment was randomly allocated to three hapa, photoperiod depended on the natural light and water temperature was monitored daily. The water quality parameters in the system were monitored weekly, the temperature ranged between 24-29°C while the concentration of dissolved oxygen ranged between 5.94-7.82 mg L<sup>-1</sup> and the pH values of the treatments ranged from 7.18-7.90. No critical values were detected for nitrite and nitrate. Two replicates of each treatment using 20 fish per hapa were reared on each of the 4 diets. The feed was manually administered and the fish were fed to satiation three times daily at 09:00 am, 12:00 pm and 16:00 pm local time. Feeding rate was subsequently adjusted according to their growth rates per hapa. The uneaten feed was siphoned out of the hapa 30 mins after each feeding period while collection of faeces samples was carried out for 2 weeks by siphoning, using a 2 cm diameter hose three hours after feeding and 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).

**Biochemical analysis:** Ten gram 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 (2000) procedures. 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 from the nitrogen content obtained by Kjeldahl method. A conversion factor of 6.25 was used for calculation of protein content according to AOAC (2000). Anti-nutritional factors of the seeds; tannins and Trypsin Inhibitor activity (TIA) were analyzed by modifying the procedures of AOAC (1984). Phytic acid was determined by the method of Latta and Eskin (1980).

**Acid insoluble ash (AIA) analysis:** AIA analyses was carried out on the diets and faeces. AIA was obtained by adding 25 mL of 10% HCl to the weighed ash content of a sample. This was covered with a water-glass and boiled

gently over a low flame for 5 min. This was then filtered using ash-less filters and washed with hot distilled water. The residue from the filter was returned to the crucible and ignited until it was carbon free after which it was weighed. Percentage AIA was calculated as:

$$\text{AIA (\%)} = \frac{\text{Weight of AIA}}{\text{Weight of Ash}}$$

**Determination of digestibility coefficient:** The determination of the protein and lipid digestibility coefficient was done according to Jimoh *et al.* (2010) which was calculated based on the percentage of AIA in feed and in faeces and the percentage of nutrient on diets and faeces.

$$\text{Apparent protein digestibility (\%)} = \frac{100 - \text{AIA in diet (\%)}}{\text{AIA in feces (\%)}} \times \frac{\text{N in feces (\%)}}{\text{N in diet (\%)}} \times 100$$

**Blood collection and haematological analysis:** Blood samples were collected in triplicate following the procedure of Klontz and Smith (1968) and Wedemeyer and Yasutake (1977) and subsequently taken to the Laboratory of the Department of Biochemistry Federal University of Technology Minna for haematological analysis. At the laboratory the clear fluid sample which is the serum was pipetted out into a clean and sterilized bottle for haematological parameters analysis (Ogbu and Okechukwu, 2001). 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 count (neutrophils and lymphocytes) were analysed as described by Dacie and Lewis (2001).

$$\text{MCV} = \frac{\text{PCV}}{\text{Erythrocytes count}} \times 10$$

$$\text{MCH} = \frac{\text{Haemoglobin}}{\text{Erythrocytes count}} \times 10$$

$$\text{MCHC} = \frac{\text{Haemoglobin}}{\text{PCV}} \times 100$$

**Evaluation of nutrient utilization parameters:** Nutrient Utilization were analyzed in terms of Feed Efficiency (FE), Specific Growth Rate (SGR), Feed Intake (FI), Protein Efficiency Ratio (PER) and Protein Retention (PR). The following formulas were used:

$$\text{Feed efficiency (\%)} = \frac{\text{Weight gained (g)}}{\text{Feed fed (g)}} \times 100$$

$$\text{Specific growth rate (\%)} = \frac{\text{In final weight (g)} - \text{In initial weight (g)}}{\text{feeding period (day)}} \times 100$$

$$\text{Feed intake (mg/fish/day)} = \text{Dry feed (mg) given/number of fish/feeding period (day)}$$

$$\text{Protein efficiency ratio} = \frac{\text{Wet body gain}}{\text{Protein intake (g)}} \times 100$$

$$\text{Protein retention (\%)} = \frac{\text{Protein gain}}{\text{Protein fed}} \times 100$$

**Statistical analysis:** Data obtained were analyzed using one-way analysis of variance (ANOVA) using Statistica 8.0 (Stat-Soft, Inc., Oklahoma, USA). Differences between treatments means were compared by Tukey’s test. Level of significance was tested at  $p < 0.05$ .

## RESULTS

Over the 8 week feeding period, no significant differences were observed in the water-quality indices between the experimental treatments. The water temperature ranged from 24.7-29.2°C; dissolved oxygen from 5.94-7.82 mg L<sup>-1</sup>; pH from 6.18-7.92 and ammonia from 0.23-0.29 mg L<sup>-1</sup>.

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%), respectively followed by soybean meal (43.63 and 7.00%), respectively, while the crude protein and lipid content of both the raw and fermented *Delonix regia* meal were (22.15-36.42 and 6.68-8.14%), respectively. Table 2 show the anti-nutritional factor composition of both the untreated Raw Flamboyant Seed Meal (RFSM) and the treated Fermented Flamboyant Seed Meal (FFSM). All the anti-nutritive factors parameters measured were lower in the treated FFSM ingredient as compared to RFSM. The proximate composition of the experimental diets is shown in Table 3. There were no much variations in the protein and crude lipid content among the experimental diets. D1 had the lowest moisture and AIA content while D3 diet moisture was higher than the other experimental diets, AIA was higher in D4 diet. Table 4 show the proximate composition of the faecal sample collected. All the nutrient values in the faecal samples were lower than the nutrient value in the diet as shown in Table 3, except for ash and the AIA.

Table 3a: Formulation of the experimental diet and proximate composition of the experimental diet for *C. gariepinus* fingerlings (g kg<sup>-1</sup>)

Ingredients	D1	D2	D3	D4
FM	529.30	473.60	417.80	362.00
SBM	100.00	100.00	100.00	100.00
FFSM	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	22.50	20.60	18.70
Cellulose	161.40	118.90	76.60	34.30
Mineral	15.00	15.00	15.00	15.00
Total	1000.00	1000.00	1000.00	1000.00
Moisture (%)	3.89	4.05	4.02	3.91
Crude protien (% d.b.*1)	38.81	38.58	38.07	38.32
Crude lipid (% d.b.*1)	8.55	8.42	8.25	8.36
Crude fiber (% d.b.*1)	4.98	5.36	5.67	5.89
Ash (% d.b.*1)	11.57	11.48	11.68	11.92
AIA (% d.b.*1)	4.22	4.35	4.25	4.58

d.b\*1: Dry bases, FM: Fish meal, SBM: Soybean meal, MM: Yellow maize, FFSM: Fermented flamboyant seed meal

Table 4: Proximate composition (%) of faecal samples of *C. gariepinus* fingerlings fed experimental diets

Ingredients	D1	D2	D3	D4
Moisture (%)	10.25	9.45	9.62	9.77
Crude protien (% d.b.*1)	9.12	9.04	9.26	9.54
Crude lipid (% d.b.*1)	3.02	3.14	3.06	3.17
Crude fiber (% d.b.*1)	3.18	3.31	3.21	3.37
Ash (% d.b.*1)	13.12	13.37	13.26	13.29
AIA (% d.b.*1)	9.75	9.65	9.82	10.12

\*d.b.: Dry bases

Table 5: Nutrient utilization and apparent digestibility coefficient of *C. gariepinus* fingerlings fed experimental diets for 56 days

Diet code	Total feed intake (g)	Feed efficiency	Protein efficiency ratio	Protein retention (%)	ADC of crude protein	ADC of crude lipid	ADC of crude fibre
D1	17.95±2.52 <sup>ab</sup>	0.85±0.01 <sup>b</sup>	2.20±0.03 <sup>b</sup>	38.81±0.51 <sup>b</sup>	89.83±2.44 <sup>a</sup>	84.71±1.83 <sup>a</sup>	72.36±0.11 <sup>b</sup>
D2	18.15±2.34 <sup>a</sup>	0.85±0.01 <sup>b</sup>	2.22±0.04 <sup>b</sup>	39.11±0.69 <sup>b</sup>	89.37±2.31 <sup>a</sup>	83.90±1.44 <sup>a</sup>	73.47±1.03 <sup>ab</sup>
D3	17.83±0.31 <sup>b</sup>	0.88±0.04 <sup>a</sup>	2.32±0.06 <sup>a</sup>	41.61±0.91 <sup>a</sup>	89.75±1.65 <sup>a</sup>	83.24±2.21 <sup>a</sup>	74.29±1.52 <sup>a</sup>
D4	18.23±3.10 <sup>a</sup>	0.84±0.01 <sup>c</sup>	2.20±0.03 <sup>b</sup>	38.72±0.44 <sup>b</sup>	88.73±3.55 <sup>a</sup>	82.84±1.22 <sup>b</sup>	74.11±1.63 <sup>a</sup>

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

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

Blood parameter	Final*1				
	Initial	D1	D2	D3	D4
PCV (%)	22.32	28.47±0.83 <sup>a</sup>	28.58±1.56 <sup>a</sup>	28.68±1.36 <sup>a</sup>	27.55±1.47 <sup>b</sup>
WBC (10 <sup>3</sup> mm <sup>-3</sup> )	7.15	7.28±0.59 <sup>a</sup>	7.34±0.23 <sup>a</sup>	7.38±0.44 <sup>a</sup>	7.57±0.36 <sup>a</sup>
RBC (10 <sup>3</sup> mm <sup>-3</sup> )	2.43	3.29±0.27 <sup>a</sup>	3.26±0.22 <sup>a</sup>	3.22±0.31 <sup>a</sup>	3.15±0.42 <sup>a</sup>
Hb (g/100 mL)	6.59	8.78±0.92 <sup>a</sup>	8.85±1.29 <sup>a</sup>	8.91±1.43 <sup>a</sup>	8.62±1.88 <sup>a</sup>
LYMPH (100)	60.32	61.95±2.83 <sup>a</sup>	62.16±3.76 <sup>a</sup>	62.48±3.66 <sup>a</sup>	61.82±4.27 <sup>a</sup>
MCHC (%)	29.53	30.84±1.34 <sup>a</sup>	30.97±1.48 <sup>a</sup>	31.07±1.87 <sup>a</sup>	31.29±1.54 <sup>a</sup>
MCH (pg)	27.12	26.69±1.52 <sup>a</sup>	27.15±1.73 <sup>a</sup>	27.67±1.92 <sup>a</sup>	27.37±1.78 <sup>a</sup>
MCV (fl)	91.85	86.53±4.84 <sup>a</sup>	87.67±3.66 <sup>a</sup>	89.07±6.55 <sup>a</sup>	87.46±3.77 <sup>a</sup>

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)

The result of nutrient utilization and diet digestibility is presented in Table 5 Fish fed D4 had the highest TFI and was significantly (p<0.05) higher and different from fish fed D3, however it was not significantly different from fish fed D1 and D2. Fish fed D3 had the highest significant FE value and was significantly different from fish fed other experimental diets (p<0.05), fish fed D4 had the lowest FE value and was significantly lower than all the fish fed the experimental diets, there was no significant difference among fish fed D1 and D2 diets in the FE value (p>0.05) however, they were significantly higher than fish fed D4 (p<0.05) and lower than fish fed D3. The PER and PR followed the same pattern, with D3 significantly higher than the other experimental diets (p<0.05) while, there was no significant difference between D1, D2 and D4. There was no significant difference in the protein digestibility among all the fish fed the experimental diets (p>0.05), however fish fed D1, D2, D3 had a higher significant lipid digestibility than fish fed D4.

The haematological composition of the initial and final groups of the fish fed with the experimental diets is given in Table 6. All the final haematological compositions were higher than the initial. Fish fed D4 diet had the lowest PCV among the fish fed experimental diets and was significantly different from those fed other experimental diets (p<0.05). While fish fed D3 had the highest PCV, it was not significantly different from those fed D1 and D2 (p>0.05). D4 had the highest WBC value among the fish fed all the experimental diets while D1 had the lowest value, however, there was no significant difference among all the fish fed the experimental diet (p>0.05). The RBC, Hb, LYMPH, MCHC, MCH and MCV followed similar pattern as WBC hence, there was no significant in all the values among all the fish fed the experimental diets (p>0.05).

## DISCUSSION

Bake *et al.*, (2013) that 20% of toasted flamboyant seed meal can be included in the diet of *C. gariepinus* fingerlings. The proximate composition of fermented *Delonix regia* meal in this present study revealed that the crude protein content was 36.42.11% (Table 2). This value was higher than the values previously reported for cooked and toasted *D. regia* meal (Bake *et al.*, 2013; 2015). This could be attributed to differences in environmental conditions such as soil types, harvesting time and also the processing method used (Bake *et al.* 2009). In this present study the Acid Insoluble Ash (AIA) was used as a marker because it has been reported to be more reliable indicator of digestibility coefficient (Halver *et al.*, 1993; Adeparusi and Jimoh 2002; Jimoh *et al.*, 2010) since, the dietary

ingredient (ash) is used and analysis of this component in faeces collected uses simple gravimetric technique. Defecation of the fish started about three hours after feeding and usually lasted for 15-30 min. This observation was in line with the observation made by Adeparusi and Jimoh (2002) on fresh water fishes. The general reduction in the nutrients of the faeces as compared to the diets as shown in Table 4, the result implies that some percentages of nutrients were absorbed and effectively utilized in the fish body. There was no significant difference ( $p > 0.05$ ) in the crude protein, lipid and ash of the faecal samples tested. This result agreed with Adeparusi and Jimoh (2002) that there was a reduction in nutrient value of faecal sample of *O. niloticus* fed lima bean (*Phaseolus lunatus*) when compared to the diets.

In this study, there was no feed rejection during the experimental period, although the acceptability and the utilization of the diets differed considerably among the treatments. This may likely be due to the levels of inclusion and palatability of the diets. This agrees with earlier reports (Riche *et al.* 2001; El-Sayed 2003; Riche and Garling Jr. 2004; Ahmad, 2008; Bake *et al.*, 2014) suggesting that when alternative protein sources especially plant protein sources are high in fish diet, palatability and attractiveness of the diets may be affected. In this study, the utilization of the experimental feeds in terms of feed efficiency, protein efficiency ratio and protein retention of improved with the inclusion of fermented flamboyant seed meal in the fish diets and the result was better compared to toasted flamboyant seed meal reported by Bake *et al.*, (2016). This could be attributed to the processing technique used which suggests that fermentation gave better result than toasting.

All the fish in this study showed normal growth and the inclusion of Fermented Flamboyant Seed Meal (FFSM) did not have any adverse effect on their morphology and nutrient utilization. Fish fed Diet 4 i.e. 30% inclusion of FFSM had the highest TFI this is an indication that 30% inclusion level of FFSM does not have any negative impact on the palatability and acceptability of the diet. This result is in line with the reports of Fagbenro (2004); Solotu and Fatoroti (2009) and Bake *et al.* (2015), who concluded that proper processing of plant protein based ingredient aid palatability and acceptability of the resulting diet provided the inclusion is at optimum level. From the result, fish fed D3 had the highest feed efficiency, protein efficiency ratio and protein retention, however fish fed D2 and D4 were not significantly different from fish fed D1 which has fish meal as its main protein source. This clearly showed that the fishes fed fermented flamboyant seed meal were able to adequately utilize the nutrients from their diets.

Protein and lipid from fermented-treated FSM meal appeared to be effectively digested by *C. gariepinus* fingerlings as those of a high quality fish meal used in this study. This would suggest that the fermentation of the flamboyant seed greatly reduced the activities of these anti-nutritional factors which aided the digestibility of the fermented product. This is in line with previous studies Bake *et al.* (2015) that proper processing of plant protein based ingredient tremendously reduces the anti-nutritional factors that limit their utilization.

Fernandes and Mazon (2003) reported that fish blood is closely related to its response to changes in the environment where it lives, natural or artificial. The responses of fish to particular stressor vary according to their characteristics, however, there are features of stress reaction common to the majority of most forms of environmental stressors which are known to alter their blood characteristics thereby leads to disruptions in metabolic activities (Ajani *et al.*, 2007), reduced growth rate and impairment of reproductive process (Mgbeka *et al.*, 2005), suppression of immune system (Auta, 2001) and in extreme cases result in mortality (Akinrotimi *et al.*, 2009).

The results of the Packed Cell Volume (PCV), Hemoglobin (Hb), Red Blood Cell (RBC) and White Blood Cell (WBC) has presented in Table 6, showed that all the haematological indices of the fish fed all the experimental diets at the end of the feeding trial increased tremendously and were higher than the initial values. This result is in line with the findings of Akintayo *et al.* (2008) Yue and Zhou (2008) Barros *et al.* (2002) Fagbenro *et al.* (2013) who reported a measurable increase in the haematological parameter observed in the fish fed test diets with respect to initial values of fish before the commencement of the feeding trial. All the values recorded in this study were within the acceptable range of a healthy juvenile cat fish (Oyelese *et al.*, 1999; Omoniye *et al.*, 2002). This indicate that FFSM was not toxic to *Clarias gariepinus* and is safe as an ingredient for aquafeed production and can be included in feed the up to 30% without any adverse effect on the fish health. The values from this study are higher than those reported by Omitoyin



(2006) when the same fish species was fed with poultry litter. Although fish fed D1 had the highest Red Blood Cell (RBC) and Hemoglobin (HB) values however, there were no significant difference between all the fish fed the experimental diets. This indicates that there was no blood lost in fish fed FFSM based diets as compared with the report of Sotulo and Faturori (2009) when *Leucaena leucocephala* was fed to *Clarias gariepinus*. D4 had the highest WBC and MCHC value although it was not significantly different from other fish fed experimental diets. This was in line with the report of Akinwande *et al.* (2004) who reported that a measurable increase in white blood count of fish or any animal is a function of immunity and animals resistance to some vulnerable illness or disease. This increase might indicate that the fish under study had high immunity or resistance to disease. Wedemeyer and Yasutake (1977) reported that blood infection might reduce haematocrit value and erythrocyte count. In this study the presence of these anti-nutritive factors were not up to a level to induce pathological changes in the fish.

## CONCLUSION

From the result obtained in this study, it is concluded that *clarias gariepinus* fingerlings can effectively digest and utilized fermented flamboyant seed meal at an inclusion level up to 30% in their diets without any adverse effect on their health and haematological indices. For effective utilization of fermented flamboyant seed meal there is need to evaluate the environmental impact of this flamboyant seed meal base diet in a long-term pond culture system.

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