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THE EFFECTS OF PROCESSED *DELONIX REGIA* SEED INCLUSION IN THE DIET OF *HETEROBRANCHUS BIDORSALIS* (GEOFFROY ST HILLAIRE 1809) FISH ON GROWTH AND NUTRIENT UTILIZATION

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Abstract

The high cost of commercial fish feed in developing countries appears to be the major problem and constraints to the aquaculture system due to competition between humans and livestock for the conventional feedstuff which has become too expensive for the fish feed producer. Therefore, this study was conducted to investigate the effects of Delonix regia seeds in two different processing methods (Fermentation and Cooking) on proximate composition and nutrient utilization of fish. Ten isonitrogenous diets (40% crude protein) were formulated using pearson square method with cooked, raw and fermented Delonix regia seed at 0% (Control), 10%, 20% and 30% inclusion, respectively. The design was a completely randomized design. Four hundred and fifty (450) Heterobranchus bidorsalis fingerlings with mean initial weight (2.64±0.01g) were stocked at fifteen fish per tank. There were significant differences (P<0.05) across the dietary treatments for proximate composition. Dietary treatments had significant (P< 0.05) effect on moisture, crude lipid, crude fibre, ash and nitrogen free extract with the exception of crude protein and dry matter in raw Delonix regia seed. All dietary treatments differed significantly (P<0.05) for moisture, crude lipid, crude fibre, ash, nitrogen free extract and dry matter respectively with the exception of crude protein in fermented Delonix regia. Moisture, crude lipid, crude fibre, ash, nitrogen free extract and dry matter differed significantly (P < 0.05) across the dietary groups with the exception of crude protein in cooked Delonix regia. Fish fed control diet had the heavier final weight (400.3 g) than the fish fed the treated diets. Protein efficiency ratio was higher in fish fed 0 % inclusion of Delonix regia seeds (9.85 %) which differs significantly (P < 0.05) from the group fed treated Delonix regia seed meal.

It is therefore concluded that inclusion of cooked Delonix regia seed up to 20% have no adverse effect on the health status of H. bidorsalis fingerlings.

Key Words: *Delonix regia seed, H. bidorsalis, processing methods, nutrient utilization, growth*

Introduction

One of the major bottlenecks faced by the fish farmers in the developing countries is the high cost of commercial feed (Lovell, 1989). According to Okoye and Sule (2001) the rapid bloom of commercial fish culture largely depends on availability of good quality and cheap feed. Fishmeal is the preferred dietary protein sources for most of the cultured fish. The limited supply, high demand and high cost has constituted a great problem to the aquaculture industry, this therefore necessitated the need to use more economical plant and animal protein ingredients in fish feed formulation that will be affordable and enhance palatable aqua feeds.

A lot of emphasis has been made on the use of plant protein sources such as; soya bean cake (Koumi *et al.*, 2009), groundnut cake (Ovie and Ovie, 2007), cotton seeds cake (El-Saidy and Gaber, 2003) and rape seed Meal (Burel et al., 2000). However, the challenge is that they are all deficient in essential amino acids, in addition the competition for these conventional plants by livestock and humans has led to their scarcity and high cost, which in turn has made them beyond the reach of aqua feed producers. Therefore, there is the need to search for alternative feedstuff which will be available and cost effective in Nigeria.

Delonix regia is endemic to the western forest of Madagascar, but has now been introduced into the tropical and sub-tropical regions worldwide. It is an ornamental legume plant noted for its fern like leaves and flamboyant display of flowers. *Heterobranchus* is one of the two main genera of family clariidae the African mud fish (*Clarias* and *Heterobranchus*) widely cultured in Africa, Asia and Europe (Adewolu and Adoti, 2010). This is due to their outstanding culture characteristics such as ability to withstand unfavorable environmental conditions, efficient in utilizing various types of locally formulated fish feed, resistance to disease, high economic potential and simple techniques in the propagation of their fingerlings (Owodeinde *et al*., 2011).

Heterobranchus bidorsalis is the second most important aquaculture species in Nigeria (Vanden Bossche and Bernacsek, 1990). However, literature on the effects of processed *Delonix regia* seeds included in the diet of *H. bidorsalis* fish on growth and nutrient utilization is very scanty. This study was therefore carried out to investigate the effect of soybean meal with inclusion of *Delonix regia* seeds at different dietary levels on growth and nutrient utilization of *H. bisorsalis* fingerlings.

Materials and Methods

The study was conducted at the aquaculture production technology unit of the skill acquisition and development centre, National Agricultural Extension and research Liason Services, Ahmadu Bello University, Zaria, located at latitude 11^{o} 09^{1} 45.2^{1} N and longitude 7^{o} 38^{1} 17.9^{1} E.

Matured and dry pods of *Delonix regia* (flamboyant) containing the seeds were collected from the annex campus of Nuhu Bamalli Polytechnic Zaria. Seeds were collected by opening the pods manually.

Fermentation of *Delonix regia* (Flamboyant) Seeds

The seeds were soaked in water for 12 hours. The drained soaked seeds were allowed to ferment naturally by tying in polythene bag and kept in a dark cupboard for 72 hours without the addition of yeast (Udensi and Okoronkwo, 2006). The fermented seeds were allowed to dry before grinding into homogenous powder using a hammer mill.

Cooking of *Delonix regia* (Flamboyant) Seeds.

The seeds were boiled at 100^{o}C for 80 mins and were allowed to dry and later grounded to homogenous powder using a hammer mill (Bake *et al.*, 2013).

Raw *Delonix regia* (Flamboyant) Seeds.

The raw seeds were dried and milled into a homogenous powder using a hammer mill.

Fish performance parameters

Weight Gain (WG)

The fish weight gain was calculated by taking the difference between final weights at the end of the experiment and the initial weight in grams (Adewolu *et al.*, 2008).

WTG (g) = Final mean weight – initial mean weight.

Specific Growth rate (SGR)

Specific growth rate was calculated using the formular as described by Aderolu *et al.,* (2010).

$$= \frac{\text{In Wf} - \text{In wi}}{t} \text{ X } 100$$

Where:Wf = Final mean weight gain (g); Wi = Initial mean weight gain (g); t = Number of days of study; In = Natural log.

Feed Conversion Ratio (FCR)

Feed conversion ratio was calculated using the formular as used by Aderolu *et al.,* (2010).

$$\text{FCR} = \frac{\text{Total feed intake (g)}}{\text{Total weight gain of fish}} \text{ X } 100$$

Protein Efficiency Ratio (PER)

Protein efficiency ratio was calculated using the formular as used by Adewolu *et al.,* (2008)

$$\text{PER} = \text{SGR} \frac{\text{Weight gain (g)}}{\text{Protein intake (g)}}$$

Statistical model

Model equation for analysis of variance used in this study

$Y_{ij} = \mu + T_i + W_{ij}$

Where: Y_{ij}= Observation of the records; μ is the effect population mean; T_i is the effect of treatments (Processing methods= Cooking and Fermentation); W_{ij} is the random effect associated with the record of the l^{th} fish

Data Analysis
Data obtained were subjected to one way analysis of variance (ANOVA) using general linear model (GLM of SAS 9.2). Duncan Multiple Range Test (DMRT) was used to test difference between levels of means and mean separation was considered significant at P<0.05.

Results
The proximate composition of raw *Delonix regia* seeds at different inclusion levels of diets (Table 1) shows the Dietary treatments had significant (P< 0.05) effect on moisture, crude lipid, crude fibre, ash and nitrogen free extract with the exception of crude protein and dry matter. Moisture content was higher in $RFSM_1$, (7.3 %), $RFSM_2$ (7.5%) and $RFSM_3$ (7.5 %), respectively and were similar (P>0.05) which differed significantly from the control group (6.6%). Control group had the highest concentration of crude lipid (11.6 %) while $RFSM_3$ had the least concentration (8.2 %). $RFSM_1$, $RFSM_2$ and $RFSM_3$ were higher in crude fibre than the control group (4.0 %). $RFSM_1$, $RFSM_2$ and $RFSM_3$ had higher concentration of ash content which differed significantly (P<0.05) from the control group. Control had the highest nitrogen free extract (29.5 %), followed by $RFSM_3$ (25.3 %) and $RFSM_3$ (23.9 %) while the least in rank is $RFSM_1$ (23.7 %).

Table 1: Mean±SD of proximate composition of raw *Delonix regia* seeds at different inclusion levels of diets

Components (%)	0% (Control)	$RFSM_1$	$RFSM_2$	$RFSM_3$	p-value	SEM
Crude protein	40.4 ± 0.68^a	40.1 ± 0.07^a	40.1 ± 1.22^a	40.1 ± 0.02^a	0.99^{ns}	0.12
Moisture	6.6 ± 0.12^b	7.3 ± 0.03^a	7.5 ± 0.02^a	7.5 ± 0.05^a	0.00	0.35
Crude lipid	11.6 ± 0.05^a	10.1 ± 0.06^b	9.6 ± 0.02^c	8.2 ± 0.03^d	0.00	1.19
Crude fibre	4.0 ± 0.01^b	9.4 ± 0.05^a	9.4 ± 0.02^a	9.4 ± 0.02^a	0.00	2.33
Ash	7.9 ± 0.03^b	9.4 ± 0.02^a	9.5 ± 0.13^a	9.5 ± 0.20^a	0.00	0.66
NFE	29.5 ± 0.02^a	23.7 ± 0.02^d	23.9 ± 0.03^c	25.3 ± 0.09^b	0.00	2.32
Dry Matter	93.4 ± 0.04^a	92.7 ± 0.01^a	92.5 ± 0.81^a	92.5 ± 0.04^a	0.43^{ns}	0.36

values are mean±SD abcd Means with different superscripts across the groups differed significantly (P<0.05).* - Significant ** - Highly significant, Ns – Not significant, NFE-Nitrogen free extract.

The proximate composition of fermented *Delonix regia* seeds at different inclusion levels of diet (Table 2). All dietary treatments differed significantly (P<0.05) for moisture, crude lipid, crude fibre, ash, nitrogen free extract and dry matter respectively with the exception of crude protein. $FFSM_3$ had higher levels of moisture (7.2 %) which differed significantly (P < 0.05) from control (6.6 %), $FFSM_1$ (6.7 %) and $FFSM_2$ (6.8 %). $FFSM_3$ had the highest concentration of crude fibre (5.34 %) which differed significantly (P < 0.05), $FFSM_2$ (5.2%), $FFSM_1$ (4.9%) and control group (4.0%), respectively. $FFSM_1$, $FFSM_2$ and $FFSM_3$ were similar (P > 0.05) across the dietary treatments but statistically significant (P < 0.05) from the control group (7.9 %) for ash content. The control group had higher concentration of nitrogen free extract (29.5 %) which differed significantly (P < 0.05) from $FFSM_1$, $FFSM_2$ and $FFSM_3$ respectively. The control and $FFSM_1$ group had higher dry matter (93.4 and 93.3 %) which differed significantly ($P < 0.05$) from $FFSM_2$ and $FFSM_3$. Highest concentration of crude lipid was recorded in the control group (11.6 ± 0.05) which differed significantly ($P < 0.05$) from other dietary groups ($FFSM_1$, $FFSM_2$ and $nFFSM_3$). $FFSM_1$, $FFSM_2$ and $FFSM_3$ was statistically similar ($P > 0.05$) among the dietary groups, through $FFSM_1$ had numerically the highest concentration of crude fibre (10.7 %) while $FFSM_3$ had the least concentration (10.6 %).

Table 2: Mean±SD of proximate composition of fermented *Delonix regia* seeds at different inclusion levels of diets

Components (%)	0% (Control)	$FFSM_1$	$FFSM_2$	$FFSM_3$	p-value	SEM
Crude protein	40.4 ± 0.68^a	40.3 ± 0.03^a	40.3 ± 0.21^a	40.2 ± 0.01^a	0.96^{ns}	0.07
Moisture	6.6 ± 0.12^b	6.7 ± 0.03^b	6.8 ± 0.01^b	7.2 ± 0.07^a	0.02	0.21
Crude lipid	11.6 ± 0.05^a	10.7 ± 0.04^b	10.7 ± 0.08^b	10.6 ± 0.02^b	0.00	0.39
Crude fibre	4.0 ± 0.05^d	4.9 ± 0.02^c	5.2 ± 0.05^b	5.3 ± 0.02^a	0.00	0.51
Ash	7.9 ± 0.03^b	9.4 ± 0.01^a	9.4 ± 0.04^a	9.5 ± 0.06^a	0.00	0.66
NFE	29.5 ± 0.02^a	27.9 ± 0.49^b	27.6 ± 0.02^b	27.3 ± 0.02^b	0.00	0.85
Dry Matter	93.4 ± 0.04^a	93.3 ± 0.07^{ab}	93.2 ± 0.02^b	92.8 ± 0.03^c	0.00	0.21

^{abcd} Means with different superscripts across the groups differed significantly ($P < 0.05$).*
- Significant ** - Highly significant, Ns – Not significant, NFE-Nitrogen free extract

Table 3 shows the proximate composition of cooked *Delonix regia* seeds at different inclusion levels of diet. Moisture, crude lipid, crude fibre, ash, nitrogen free extract and dry matter differed significantly ($P < 0.05$) across the dietary groups with the exception of crude protein. $CFSM_2$ (6.9%) and $CFSM_3$ (6.9 %) had higher concentration of moisture which differed significantly ($P < 0.05$) from $CFSM_1$ (6.7 %) and control group (6.6%). The control group had the highest concentration of crude lipid (11.6 %) which differed significantly ($P < 0.05$) from $CFSM_1$, $CFSM_2$ and $CFSM_3$ respectively, though $CFSM_2$ (10.2 %) and $CFSM_3$ (10.1%) was statistically similar ($P>0.05$). $CFSM_2$ and $CFSM_3$ had the highest crude fibre content and were statistically similar ($P>0.05$) but differed significantly ($P < 0.05$) from $CFSM_1$ (4.3 %) and control group (4.0%). $CFSM_3$ had the highest ash content (9.5 %) which differ significantly ($P < 0.05$) from $CFSM_2$ (9.3 %), $CFSM_1$ (9.1 %) and control (7.9 %). The control group had the highest levels of nitrogen free extract (29.5 %) which differed significantly from $CFSM_1$, $CFSM_2$ and $CFSM_3$ respectively. $CFSM_1$ and $CFSM_2$ were statistically similar ($P > 0.05$). The control group and $CFSM_1$ had the highest dry matter (93.4 and 93.3 %) which differs significantly ($P < 0.05$) from $CFSM_2$ (93.1 %) and $CFSM_3$ (93.1 %) respectively.

Table 3: Proximate composition of cooked *Delonix regia* seeds at different inclusion levels of diets

Components (%)	0% (Control)	$CFSM_1$	$CFSM_2$	$CFSM_3$	p-value	SEM
Crude protein	40.4 ± 0.68^a	40.2 ± 0.01^a	40.2 ± 0.03^a	40.1 ± 0.03^a	0.96^{ns}	0.39
Moisture	6.6 ± 0.12^b	6.7 ± 0.03^b	6.9 ± 0.03^a	6.9 ± 0.03^a	0.02	0.14
Crude lipid	11.6 ± 0.05^a	10.6 ± 0.03^b	10.2 ± 0.03^c	10.1 ± 0.04^c	0.00	0.58
Crude fibre	4.0 ± 0.01^c	7.3 ± 0.02^b	7.3 ± 0.02^a	7.4 ± 0.03^a	0.00	1.43
Ash	7.9 ± 0.03^d	9.1 ± 0.03^c	9.3 ± 0.03^b	9.5 ± 0.01^a	0.00	0.59
NFE	29.5 ± 0.02^a	26.2 ± 0.05^b	26.1 ± 0.02^b	26.0 ± 0.01^c	0.00	1.46
Dry Matter	93.4 ± 0.04^a	93.3 ± 0.03^a	93.1 ± 0.03^b	93.1 ± 0.01^b	0.00	0.14

^{abcd} Means with different superscripts across the groups differed significantly ($P<0.05$).*
- Significant ** - Highly significant, Ns – Not significant, NFE-Nitrogen free extract

Table 4 shows the growth performance, feed intake and survival rates of *H. bidorsalis* fed processed *Delonix regia* seeds at different inclusion level of diet. All the performance and feed utilization characteristics differs significantly ($P < 0.05$) among the dietary treatments with the exception of initial weights. Fish fed control diet had the heavier final weight (400.3 g) than the fish fed the treated diets as shown in Figure 1. Fish fed raw *Delonix regia* seed meal at 30 % inclusion level had the lowest final weight (222.0 g). The control group had higher weight gain (397.7 g) which differs significantly ($P < 0.05$) across the dietary treatments.

The least weight gain was recorded in the fish fed raw *Delonix regia* seed meal at 30% inclusion (219.3g). Fish fed the control diet had the highest specific growth rate which differs significantly from other dietary treatments. Also, R_{30} recorded the weakest specific growth rate. C_{10}, C_{20}, C_{30} and F_{10} groups had similar ($P > 0.05$) specific growth rate (2.7, 2.7, 2.6 and 2.6g) respectively which differs significantly from F_{20}, F_{30}, R_{10}, R_{20} and R_{30} respectively. Protein efficiency ratio was higher in fish fed 0 % inclusion of *Delonix regia* seeds (9.85 %) which differs significantly ($P < 0.05$) from the group fed treated *Delonix regia* seed meal. The number of fish stocked were similar (n=45) across the dietary treatment. All the fish stocked were all harvested in the control, C_{10} and C_{20} groups with 100 % survival rates. C_{30} and F_{10} groups had 98% survival rate with forty-four (44) fish harvested, while F_{20} and F_{30} recorded 96% survival rate, though R30 recorded the least survival rate (84.0 %) as shown in Figure 2. All the dietary groups were statistically similar ($P > 0.05$) across the treatments, with the exception of R_{30}.

Table 4: Growth performance, feed utilization and survival rates of *Heterobranchus bidorsalis* fed processed *Delonix regia* seeds at different inclusion levels of diet

Parameters	0%	$CFSM_1$	$CFSM_2$	$CFSM_3$	$FFSM_1$	$FFSM_2$	$FFSM_3$	$RFSM_1$	$RFSM_2$	$RFSM_3$	SEM	p-value
MIW(g)	2.60^a	2.68^a	2.60^a	2.64^a	2.65^a	2.60^a	2.64^a	2.69^a	2.60^a	2.68^a	0.01	0.40
MFW(g)	400.3^a	360.6^a	341.1^c	329.7^d	331.1^d	310.4^e	286.7^f	266.6^g	241.1^h	222.0^i	2.97	0.00
MWG(g)	397.7^a	357.9^b	338.5^c	327.1^d	328.5^d	307.8^e	284.1^f	263.2^g	238.5^h	219.3^i	2.93	0.00
MFI(g)	1226.3^a	1052.1^b	993.5^c	908.2^c	932.4^d	876.2^f	831.4^g	769.2^h	711.8^i	659.7^j	0.05	0.00
SGR(%)	2.75^a	2.68^b	2.66^b	2.64^b	2.64^b	2.61^c	2.56^d	2.51^e	2.48^e	2.41^f	4.56	0.00
FCR	3.08^g	2.94^d	2.94^d	2.78^a	2.84^b	2.85^b	2.93^d	2.91^c	2.98^e	3.01^f	0.02	0.00
PER	9.85^a	8.91^b	8.43^c	8.15^d	8.15^d	7.64^e	7.07^f	6.58^g	5.95^h	5.47^I	0.02	0.00
NFS	45^a	45^a	45^a	45^a	45^a	45^a	45^a	45^a	45^a	45^a	0.21	0.00
NFH	45^a	45^a	45^a	44^a	44^a	43^b	43^b	42^c	41^d	38^e	1.00	0.00
SR (%)	100^a	100^a	100^a	98.0^a	98.0^a	96.0^a	96.0^a	93.0^a	91.0^a	84.0^b	5.36	0.04

^{abcdefghi} Means with the different superscripts differ significantly (P<0.05) across the treatments. Mean initial weight, MFW-Mean final weight, MWG-Mean weight gain, MFI-Mean feed intake, SGR-Specific growth rate, FCR-Feed conversion ratio, PER-Protein efficiency ratio, NFS-No. of fish stocked, NFH- No. of fish harvested, SR-Survival rate

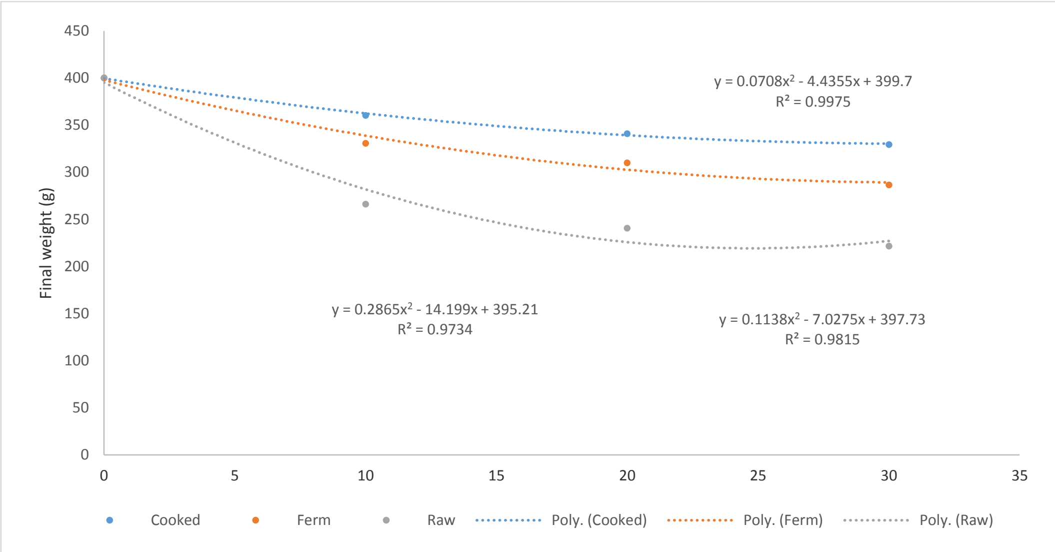

Figure 1: Effect of varying inclusion levels of differently procesed D.regia seeds on final weight of H.bidorsalis

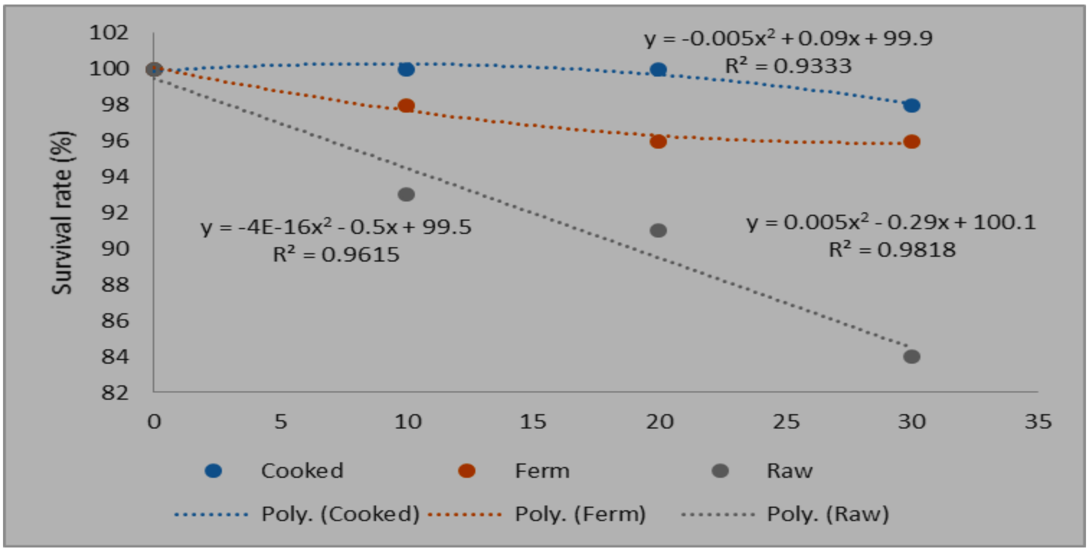

Figure 2: Effect of varying inclusion levels of differently procesed D.regia seeds on survival rate of H.bidorsalis

DISCUSSION

The range of values for moisture, crude protein and lipid were higher than the values obtained by Balogun *et al*. (2004) for moisture, crude protein, and lipid content, ash, and dry matter content. It was also lower than the range of values reported by Balogun *et al*. (2004) for NFE and dry matter. The values of moisture, crude protein and lipid in this study was higher than the values of 3.7 and 3.2 %; moisture, 23.2 and 25.1%; crude protein, 4.6 and 7.3 %; lipid, and 8.8 and 6.5 %; ash in the raw *Delonix regia* seed meal and cooked *Delonix regia* seed meal as reported by Bake *et al*. (2013). Weight gain and standard length are biometric indicators for measuring fish responses in experimental designs (Balogun *et al*., 2004). Differences among the experimental diets agree with the findings of some researchers (El- Sayed 1999; Bake *et al*., 2009). This could be linked to texture, palatability and taste of experimental diets and are also related to the processing methods. The best growth response observed in fish fed 10 % inclusion of cooked *Delonix regia* seeds ($CFSM_1$) agrees with the trend observed by Bake *et al*. (2013) in the nutritional evaluation of varying levels of cooked *Delonix regia* seed meal on the growth performance and body composition of Nile Tilapia fingerlings in Nigeria. The high feed intake in C_{10} in this study could also be connected to the cooking technique employed in the processing of flamboyant seed. This may have reduced the effect of antinutritive factors and other secondary compound activities in the seed. The weight gain and specific growth rate increase in the fish fed cooked *Delonix regia* seed meal than other dietary group could be linked to the variations in amino acid composition of the diets, digestible energy and environmental conditions (Olufeagba *et al*., 2002). The mean feed conversion ratio of 2.78 to 3.08 for *H. bidorsalis* in this study compared favourably with those of *H. bidorsalis* and *C. gariepinus* fingerlings (Reginald 2013), *Heterobranchus bidorsalis* fry (Dada *et al*., 2001) and *C. gariepinus* fed fermented shrimp head waste meal (Nwanna, 2003).

The high specific growth rate values (SGR), high feed conversion ratio (FCR), protein efficiency ratio (PER), final weight gain, feed intake and survival rate of fish fed cooked *Delonix regia* seed meal at 10 % inclusion levels confers it with better advantages for growth and efficiency of feed utilization over the rest of the experimental diets. The result agrees with the report of Olaniyi *et al.* (2009) who stated that the higher the specific growth rate (SGR) and the smaller the feed conversion ratio (FCR), the better the feed quality.

Adikwu (2003) also reported that a lower feed conversion ratio implies efficient feed utilization by fish. The results further indicated inconsistent growth indices in the fish fed the experimental diets as reflected in the survival rates. This could be attributed to the differences in the processing methods and variations in the inclusion levels of the processed seeds in the diets. Significant differences observed in the survival rates could be linked to the antinutritional factors in the feed samples that might have resulted in low acceptability and palatability which could finally result into death. Trend observed in this study is connected to the report of Pompa (1982) and NAERLS (1991) who reported that high anti- nutrient and low palatability could result in low feed consumption and utilization.

CONCLUSION

- This study showed that processing methods showed significant variations across the dietary treatments.
- Cooking at different inclusion levels compared favourably in biological performance to the control for survival rate and final weight gain.
- Inclusion of cooked *Delonix regia* in diets upto 20% is suitable for optimum performance of *Heterobranchus bidorsalis* fish

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