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Growth performance and hematology indices of *Clarias gariepinus* (Burchell, 1822) fed graded level of roasted *Jatropha curcas* meal

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Abstract

This study was aimed at evaluating the effect of replacing roasted *Jatropha curcas* (*J. curcas*) meal with maize on performance and hematology indices of *Clarias gariepinus* (*C. gariepinus*). Four diets containing varied inclusion of *J curcas* replacing maize at 0% (D1), 10% (D2), 20% (D3) and 30% (D4) were formulated. *C. gariepinus* (n=180) weighing 29.52±0.40g were fed to satiation with the treatments for 12 weeks. Each treatment was in triplicate. Roasted *J. curcas* had 12.37% crude protein and 31.83% carbohydrate. Supplementation of *J. curcas* significantly (P<0.05) increased final weight gain of *C. gariepinus* fed 10% (68.96±3.53) *J. curcas* replaced diet and least in diet D3. *C. gariepinus* had least feed conversion ratio when fed diet D2 (2.23±0.67). However, Packed Cell Volume (PCV) of the experimental fish were unaffected with *J. curcas* supplementation and the values ranged from D4 (16.67±2.08) to D1 (22.33±3.05). Red blood cell (RBC) significantly reduces as *J. curcas* supplementation increases with the higher RBC in diet D1 (2.27±0.21) and least in diet D4 (1.77±0.31). *C. gariepinus* fed diet D2 had a significantly higher whole body crude protein value of 62.65±0.58 and *C. gariepinus* fed diet D4 had the least value. Supplementation of *J. curcas* in *C. gariepinus* could improve growth performance and health status with 10% *J. curcas* inclusion and any further supplementation could negatively affect performance and health of the fish.

Keywords: *Jatropha curcas; Clarias gariepinus*; Growth performance; Hematology

1 Introduction

In Nigeria, high cost of fish feed production has posed a lot of threat to aquaculture development due to the ever-increasing cost of fish feed which resulted in an increase in cost of fish production. Fish feed contributed to about 40-70% of the variable cost of commercial aquaculture operation for many fish species such as *Clarias gariepinus* (*C. gariepinus*) [1,2]. However, it is driven by the high cost of feed ingredients such as maize, an important feed source in fish feed. Therefore, the need to identify alternative source of carbohydrate (energy) to develop low-cost feed ingredients on the basis of sustainable and renewable feed resources for small and medium scale fish farmers are therefore crucial. It is also important to consider the selected feed ingredients that do not conflict with human food security interest in which *Jatropha curcas* (*J. curcas*) is one because of its high carbohydrate level after been processed and is readily available with less competition with human consumption.

However, *J. curcas* is anticipated to be one of the potential plant species to be a cheap source of food stuff for fish diet. *J. curcas* is a multipurpose plant species which could be used for bio-fuel and bio-diesel. It is widely distributed in many tropical and subtropical countries. The processed seed is referred to as Jatropha seed meal. The use of Jatropha seed meal was impeded so far, because of its toxicity, which is mainly ascribed to the presence of phorbol esters (PEs) [3]. The meal also contains antinutritive factors such as trypsin inhibitors, lectin and phytate [4]. Since these anti nutrients

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are heat labile, roasting method could be a suitable processing method to detoxify Jatropha seed meal. However, [5] reported that heat treatment followed by solvent extraction could remove phorbol esters and will result in elimination of most of the anti-nutrients and toxins from the toxic variety.

Furthermore, several plant by-products has been used to replace maize which is a source for energy in *C. gariepinus* diets such as Toasted African breadfruit seed meal [6], *Chysophyllum albidum* [7], Sweet potato peel [8], Soak plantain peel [9] and Melon seed peel [10]. However, information on the growth performance and hematological indices of *C. gariepinus* fed roasted Jatropha meal as a replacement of maize which is energy source is limited. Therefore, this study is aimed at evaluating the effect of replacing maize with roasted *Jatropha curcas* (*J. curcas*) meal on performance and hematology indices of *C. gariepinus*.

2 Material and methods

2.1 Experimental Site

The experiment was carried out at the aquaculture laboratory unit of Joseph Ayo Babalola University, Ikeji-Arakeji, Osun state, Nigeria.

2.2 Feed ingredient and diet formulation

The *Jatropha curcas* seed were obtained from ripe *J. curcas* fruits harvested from different locations in Ado-Ekiti, Ekiti State, Nigeria. *Jatropha curcas* were sorted, sundried, dehulled and roasted with sand. The sand was heated up to 95^{0}C before the introduction of *J. curcas* seed. The sorted seed was introduced into the heated sand and roasted for thirty minutes at 110 ^{0}C, while it was continually stirred to allow even roasting. The roasted seed was later milled and defatted mechanically with hammer grinder. Processing was according to [11]. The basal feed ingredients were obtained from a reputable fish feed mill in Akure, Ondo state. The ingredient was thoroughly mixed in different proportion with roasted *J. curcas* used to replace yellow maize at 0, 10, 20 and 30% as described in table 1. After this, all the four treatment including control diet without roasted *J. curcas* were pelleted in a mechanical pelleting machine. The pelleted diets were sundried to reduce the moisture content and kept in air tight containers prior to the experiment.

2.3 Experimental design and feeding regime

180 pieces of *C. gariepinus* with an average weight of 29.52±0.40 g were randomly distributed into 12 experimental tanks for four treatments in triplicates. Prior to distribution of fish into treatment, the fish were acclimatized for 14days until they became active and they were fed locally available feed. Each tank were filled with 15000 mg of unchlorinated water and stocked with 15 pieces of fish. The fish were fed diet containing graded levels of *J. curcas* meal for six weeks (42 days). The experimental fish were fed two times daily at the rate of 5% body weight [12]. Feedings were generally done in the morning at 09:00 and 15:00h except on sampling days when they are fed after weighing. During the experimental period, the amounts of feed were adjusted weekly based on the body weight of the fish for the subsequent weeks. Faeces and uneaten feed residues were siphoned out of the experimental tanks daily, while the water in each tank were changed every two days.

2.4 Diet formulation and preparation

Experimental diets were formulated with roasted *J. curcas* meal in replacing maize on a dry basis to maintain the crude protein and energy levels. Four iso-nitrogenous and iso-energetic diets were formulated to evaluate the nutritive value of catfish *C. gariepinus* juvenile. The roasted Jatropha meal was included in the diet at levels of 0, 10, 20 and 30%. The diet containing 0% roasted Jatropha meal serves as the control. The ingredients were milled using milling machine and mixed together before the addition of vitamin premix. Warm water was added to the pre-mixed ingredients and homogenized until a dough-like paste was formed. The dough was then passed through a pelleting machine. The moist pellets were sundried to a constant weight and kept in air tight containers prior to the experiment. The percentage composition of the experimental diets is given in Table 1.

2.5 Data collection and analysis

During the experiment, body weights of the fish were measured every week. Feeding behaviour of the fish was also monitored throughout the experiment.

2.6 Weight Gain (WG)

$$\text{Weight gain} = \text{Final Body Weight} - \text{Initial Body Weight}$$

2.7 Specific Growth Rate (SGR %)

$$SGR = \frac{\text{Logc W2} - \text{LogcW1}}{\text{T2} - \text{TI 1}} \text{ x 100}$$

Where W_1 = Initial weight of fish (gm), W_2 = Final weight of fish (gm), T_2 = Time T_2,

2.8 Feed Conversion Ratio (FCR)

$$FCR = \frac{\text{Feed Consumed}}{\text{Weight gain}}$$

2.9 Feed Efficiency (FE)

$$FE = FCR \times \frac{\text{Feed crude protein content \%}}{\text{Cultured species crude protein content \%}}$$

2.10 Hematological analysis

Blood (1-3 mL of fish) was collected from the fish on the last day of the feeding trial. Collection of blood was done through the vertebral caudal vessel with the help of disposable hypothermic syringe and needle. Blood was emptied into 10ml sample bottle treated with anti-coagulant, Ethylene Diamine Tetracetic Acid (EDTA). Hematological analysis of fish followed the method described by [13]. Blood cell counts (erythrocytes and leucocytes) was carried out in an improved Neubaeurhaemocytometer using a modified Yokoyama diluting fluid. The erythrocyte indices, mean cell haemoglobin concentration (MCHC) were computed from haemoglobin values and erythrocyte counts.

2.11 Proximate analysis

The proximate analysis was carried out based on the standard of Association of Analytical Chemists [14].

2.12 Statistical analysis of data

Table 1 Gross composition of roasted *Jatropha curcas* meal

Ingredients %	ControlD1 (0%)	D2 (10%)	D3 (20%)	D4 (30%)
Maize	37.00	33.30	29.60	25.90
Jatropha (RJM)	0.00	3.70	7.40	11.10
Groundnut cake	15.00	15.00	15.00	15.00
Fish meal (72%)	22.00	22.00	22.00	22.00
Soya bean meal	17.00	17.00	17.00	17.00
Wheat offal	8.00	8.00	8.00	8.00
Lysine	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10
Vit. C	0.35	0.35	0.35	0.35
Multi-Enzyme	0.10	0.10	0.10	0.10
Salt	0.15	0.15	0.15	0.15
Vitamin premix	0.10	0.10	0.10	0.10
Dicalcium phosphate	0.10	0.10	0.10	0.10
TOTAL	100	100	100	100

Note: D1(0%) - 0% inclusion of *Jatropha curcas*; D2 (10%) – 10% *Jatropha curcas*; D3(20%) - 20% *Jatropha curcas*; D4 (30%) – 30% *Jatropha curcas*

Data were subjected to descriptive statistics and one way analysis of variance [ANOVA] using 16.0 version of SPSS statistical package. Means were separated using the Duncan's multiple range tests of the same software at $\alpha_{0.05}$.

Table 2 Proximate Composition of the roasted *Jatropha curcas* meal

Nutrients	Percentage (%)
Moisture	9.8
Ash	2.64
Fat	34.27
Fiber	9.09
Protein	12.37
Carbohydrate	31.83

3 Results

Table 3 shows growth performance of *C. gariepinus* fed roasted *J. curcas* meal. Supplementation of *J. curcas* had a significant influence (P>0.05) on the growth performance of *C. gariepinus*. Fish fed diet D_2 had a significantly higher (P>0.05) value of 68.96±3.53g in the final weight than other diets. The weight gain of fish fed diet D_2 (39.72±0.02g) differ significantly from other diets, while the least values was recorded in diet D_3. Also, similar trend was observed in Specific growth rate as noted in weight gain with the highest value of 20.55±0.05 in fish fed diet D_2. However, *C. gariepinus* fed diet D_2 (2.23±0.03) had the least feed conversion ratio and higher value in diet D_3. Supplementation of *J. curcas* had no influence on the feed efficiency.

Table 3 Growth performance of *C. gariepinus* fed varied inclusion levels of roasted *J. curcas* meal

Parameters	ControlD1 (0%)	D2 (10%)	D3 (20%)	D4 (30%)
Initial weight (g)	29.48	29.24	29.84	29.52
Final weight (g)	67.55±5.11a	68.96±3.53b	64.22±6.00a	65.73±2.64a
Weight gain (g)	38.07±0.02c	39.72±0.02d	34.38±0.02a	36.21±0.01b
Specific growth rate (g)	16.90±0.10c	20.55±0.05d	12.05±0.05a	15.88±0.08b
Feed Conversion Ratio (FCR)	2.50±0.30ab	2.23±0.03a	2.69±0.05b	2.30±0.20b
Feed Efficiency (FE)	0.40±0.20a	0.45±0.05a	0.37±0.04a	0.43±0.03a

Note: D1(0%) - 0% inclusion of *Jatropha curcas*; D2 (10%) – 10% *Jatropha curcas*; D3(20%) - 20% *Jatropha curcas*; D4 (30%) – 30% *Jatropha curcas*

Proximate composition of *C. gariepinus* whole body varied significantly with varied inclusion of roasted *J. cucas* meal in fish diet as shown in Table 4. Crude protein of fish fed diet D_2 had a significantly higher values of 62.65±0.58% and least value in diet D_3 and D_4. Fat content reduces as the inclusion level of *J. curcas* increases. However, ash content increases as *J. curcas* increases but the value dropped at 30% (D_4) inclusion level. Moisture content varied significantly with the higher value in fish fed diet D_4 (44.62±0.36%) and least value in D_3 (43.63±0.84%). Carbohydrate value of fish fed diet D_2 had the least value but varied significantly from other diets.

Table 4 Proximate composition of *C. gariepinus* whole body fed varied inclusion levels of roasted *J. curcas* meal

Parameters	Control D_1 (0%)	D_2 (10%)	D_3 (20%)	D_4 (30%)
Protein	62.28±1.68^{b}	62.65±0.58^{c}	61.12±0.74^{a}	60.93±2.72^{a}
Fat	9.30±0.57^{c}	7.51±0.99^{ab}	8.01±0.64^{b}	6.71±1.07^{a}
Ash	6.39±1.31^{a}	7.51±0.99^{ab}	8.01±0.64^{b}	6.71±1.07^{ab}
Moisture	44.53±0.91^{c}	43.93±0.69^{b}	43.63±0.84^{a}	44.62±0.36^{d}
Carbohydrate	7.49±1.68^{b}	6.12±2.26^{a}	8.37±0.78^{c}	8.93±1.35^{d}

Note: D1(0%) - 0% inclusion of *Jatropha curcas*; D2 (10%) – 10% *Jatropha curcas*; D3(20%) - 20% *Jatropha curcas*; D4 (30%) – 30% *Jatropha curcas*

Hematology Indies of *C. gariepinus* fed varied inclusion levels of roasted *J. curcas* meal as shown in Table 5. Varied inclusion of roasted *J. curcas* meal in *C. gariepinus* diet significantly reduced the PCV values with the highest value (22.33±3.05%) observed in control diet and least in fish fed diet D4. WBC of fish fed diet D2 had the significantly higher value than other diet. For RBC, *C. gariepinus* fed diet D1 and D3 were not significantly different from each but were higher than other treatments. Fish fed diet D4 had significantly higher values for MCH and MCHC and least in diet D3. Diet D2 and D3 had higher values of MCV. Neutrophil values significantly reduce as the level of roasted *J. curcas* increases. Varied inclusion of roasted *J. curcas* had on significant (P<0.05) effect on lymphocytes. M values varied significantly with varied roasted *J. curcas* inclusion in *C. gariepinus* diet.

Table 5 Hematology Indies of *C. gariepinus* fed varied inclusion levels of roasted *J. curcas* meal

Parameters	Control D_1 (0%)	D_2 (10%)	D_3 (20%)	D_4 (30%)
PCV (%)	22.33±3.05^d	19.33±2.08^b	21.00±6.08^c	16.67±2.08^a
Hb (g/mm^3)	7.73±0.73^d	6.63±0.55^b	7.17±2.02^c	6.26±0.64^a
WBC (10^4/mm^3)	1757.33±261.9^b	1993.67±163.3^d	1524.33±18^a	1858.67±182^c
RBC (10^6/mm^3)	2.27±0.21^c	2.10±0.20^b	2.28±0.64^c	1.77±0.31^a
MCH (pg)	34.13±1.97^c	31.63±1.25^b	9.53±2.75^a	36.50±2.83^d
MCHC (T/T)	34.73±1.55^c	34.33±0.96^b	34.13±0.49^a	37.63±0.91^d
MCV (μ)	8.73±0.93^b	9.53±2.75^c	9.53±2.75^c	7.57±0.92^a
N	11.33±5.03^c	10.67±3.79^c	7.33±1.52^b	5.00±1.73^a
L	86.33±6.87^a	88.00±4.00^a	89.00±1.00^a	94.00±2.00^b
M	3.50±0.71^c	2.00±1.41^b	3.67±2.08^c	1.50±0.71^a

Note: D1(0%) - 0% inclusion of *Jatropha curcas*; D2 (10%) – 10% *Jatropha curcas*; D3(20%) - 20% *Jatropha curcas*; D4 (30%) – 30% *Jatropha curcas*

4 Discussion

Growth performance of *C. gariepinus* fed varied inclusion levels of roasted *J. curcas* meal as shown in Table 3. Varied inclusion of roasted *J. curcas* meal significantly affected the growth performance of the experimental fish. The growth performance of *C. gariepinus* fed roasted *J. curcas* meal decreases as the inclusion level increases in the diet as observed in final weight, weight gain and specific growth rate. Fish fed 10% inclusion had the highest values when fed varied inclusion of roasted *J. curcas* meal than the control and other diets. The decrease observed in this present study as the level of inclusion increases could be as a result of the presence of anti-nutritional factor. Also, this could also be as an indicator of poor palatability of the ingredient as the inclusion level of roasted *J. curcas* meal increases in the diet. [7] reported similar trend when *Chrysophyllum Albidum* based diet was fed to *C. gariepinus*. [7] attributed the reduced feed intakes and weight gain by *C. gariepinus* with the increasing inclusion of the test ingredient as a result of palatability which could result from the high presence of anti-nutritional factors in the diet. [15] & [16] reported consistent decrease in percentage weight gain in plantain peel meal and sweet potato peel meal inclusion in the experimental diets, respectively. Furthermore, this finding agrees with [6], who observed decrease in growth performances as toasted African breadfruit inclusion levels increased in the experimental diet. [6] attributed it to the presences of anti-nutritional factors which could be responsible for reduced palatability and lower feed intake recorded in their study. On the contrary, [17, 10] reported that Potato peel and melon seed peel as dietary carbohydrate source improved growth performance of *Oreochromis niloticus*, respectively.

The result of this findings showed that *C. gariepinus* fed *J. curcas* meal response positively by enhancing protein deposition in the carcass of the fish as observed in Table 4. Fish fed diet D2 (10%) had the highest crude protein. It could be ascribed to the fact that poised amino acid profile stimulated protein synthesis in the muscle while supplemental amino acid levels above what is required for growth does not improve deposition due to increased rate of protein catabolism [18]. Also, crude fat content and carbohydrate reduced as inclusion *J. curcas* increased. This could be attributed to the fact that reduced deposition of fat in fish therefore increases the carcass quality of fish.

Health status, metabolic disorders and chronic stress status either before or after clinic examination of specimens are indicators that provide more information about haematology study [19, 10]. However, the haematology indices of *C. gariepinus* fed varied inclusion levels of roasted *J. curcas* meal varied significantly as shown in Table 5. Reduction in the

PCV and Hemoglobin of the experimental fish reduced significantly with the supplementation of roasted *J. curcas* meal in fish diet. Reduction in the level of PCV observed in this study could have increase the manifestation of anemia in the experimental fish. This could have resulted in the reduced final weight of the experimental fish as observed in growth performance study. This means that decrease in PCV as the level of *J. curcas* increases might not provide an effective platform for blood oxygen transporter system which could result to inadequate utilization of nutrient. [20] Reported that hemoglobin values could be an indicator to diagnosed anaemia because hemoglobin plays vital role in carrying capacity and oxygen-binding of the blood. In this present work, inclusion of *J. curcas* meal beyond 10% inclusion could increase the risk of anaemia in fish. [10] However had a contrary result when dietary inclusion levels of melon seed peel was fed to *Oreochromis niloticus* and reported that feeding *Oreochromis niloticus* up to 100% and 75% inclusion of melon seed peel improved PCV and Hemoglobin. Also, increase in PCV, Hemoglobin, RBC and WBC values was observed in *C. gariepinus* fed different levels of melon peel diet [21]. [22] noted a significant increase in RBC when agro-waste was used to replace maize as carbohydrate source in *C. gariepinus*.

Inclusion of *J. curcas* in fish diet increases the RBC and WBC when fed 10% inclusion level but reduces as the inclusion level increases. [20] informed that an increase in RBC could result from the improvement observed in the carrying capacity of fish blood as a result of the discharge of new RBCs from the erythropoietic tissue. WBC performs a significant role in fighting infection and reactions of living organism. Therefore, increased WBC shows that the experimental fish fed 10% inclusion of *J. curcas* could boost immune system and normal physiological reactions under toxic condition

5 Conclusion

The present study has shown that supplementation of maize with roasted *J. curcas* as a source of energy in *C. gariepinus* diet could improve growth performance, increase protein deposition in fish flesh and better the health status at 10% inclusion levels. Therefore, 10% inclusion of roasted *J. curcas* meal is recommended to replace maize in fish diet. Any replacement of *J. curcas* beyond 10% inclusion level will have adverse effect on the growth and health status of the fish.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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