

# *Cascabela thevetia* seed meal as a dietary alternative protein for *Clarias gariepinus* fingerlings

Theophilus Olayiwola Babalola, Bisola Bridget Alabi, John Bunmi Olasunkanmi, Jeremiah Olanipekun Jimoh

Department of Fisheries and Aquaculture, Federal University, Oye-Ekiti, Nigeria

**Correspondence Author:** Theophilus Olayiwola Babalola, Department of Fisheries and Aquaculture, Federal University, Oye-Ekiti, Nigeria  
E-mail address: theophilus.babalola@fuoye.edu.ng

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## ABSTRACT

The rising cost of conventional protein sources in aquafeed has necessitated the search for alternative that is cheap, readily available, and not in competitive use with human and other livestock. This study evaluates the efficacy of replacing soybean meal with detoxified *Cascabela thevetia* seed meal (CTSM) on growth performance, tissue composition and haematology of *Clarias gariepinus*. *Clarias gariepinus* fingerlings (N=150) were assigned to varying levels of *Cascabela thevetia* seed meal (CTSM) namely 0, 50, 100, 150, or 200 g/kg diet and designated as CTSM0, CTSM50, CTSM100, CTSM150 or CTSM200 respectively as diet treatments. Ten fish were batch weighed into each of the fifteen tanks used for the feeding trial and the tanks were randomly assigned to each dietary treatment in triplicates. Fish were fed 5% of their body weight two times daily for 56 days. Fish growth performance was not significantly different ( $P>0.05$ ). However, the tissue composition and haematological parameters were significantly different ( $P<0.05$ ). Fish fed diet CTSM200 had the highest growth performance values compared to the other treatments and control. PCV, Hb, WBC, MCV and MCH were found to be highest in fish fed diet CTSM200. Neutrophil, lymphocyte and MCHC were not influenced by the dietary treatments. The results indicate that CTSM can be successfully incorporated in the diet of fish without serious negative impacts on growth performance and health. 20% CTSM dietary inclusion level resulted in highest growth performance, it is therefore recommended in the diet of *C. gariepinus*.

**Keywords:** alternative feedstuff, *Clarias gariepinus*, growth performance, hematology, *Cascabela thevetia*.

## INTRODUCTION

Fish is a relatively cheap source of animal protein in the human diet. Fish is also a good source of vitamins, minerals, fatty acids, and other micronutrients essential to a healthy diet (Babalola, 2010). However, the availability of these nutrients to humans will be made possible if the cost of production is reduced. The cost of production can be reduced if conventional protein (fishmeal, soybean meal, groundnut cake) feedstuffs, which are scarce, expensive and in competitive use with humans and other livestock, are replaced partially or wholly by non-conventional feedstuffs.

Many researchers have made attempts on the utilization of non-conventional feedstuffs as alternative protein sources in the diets of fish. (Babalola et al., 2020, Adebayo 2017, Fadel et al., 2017). One of the promising underutilized plant protein sources used in animal diets is *Cascabela thevetia*, a member of the family Apocynaceae and commonly called yellow oleander, lucky nut tree, be-still tree, and milk bush (Ibiyemi et al., 2002). *C. thevetia* is mainly grown as an ornamental tree in gardens and along roadsides. Presently, there is no human use for it as a dietary ingredient, making it cheap compared to other conventional protein concentrates (Taiwo et al., 2004). *C. thevetia* can grow in harsh conditions (Ibiyemi et al., 2002). It can thrive in both rain and dry conditions or where there is no water. It requires no fertilizer application and fruits profusely. Divergent reports of the protein contents of *C. thevetia* seeds are available. According to Nair et al. (1982) the seed contains about 35% protein, Ibiyemi et al.

(2002) and Ofoegbu and Kelle (2013), in a different study, reported 37% crude protein in the seeds. However, Atteh *et al.* (1995) said 47.5% protein in the seed. Similarly, from the Oluwaniyi *et al.* (2007) report, the crude protein content of the defatted seed ranged from 42.79 – 47.50% and crude lipid from 4.40 to 4.80%. *C. thevetia* seed can be made into a cake and used as a protein supplement in livestock diet if the oil, which constitutes about 40-60 %, is appropriately extracted.

The utilization of the seed in animal feed has been hampered because of the presence of anti-nutritional factors such as cardiac glycosides, phenols, terpinoids, oxalates, phytic acid and saponins (Orji and Okfor 2000; Bandara *et al.*, 2010). Glycosides have been extracted from *C. thevetia* plant, and the major one is thevetin which is responsible for the bitterness and low palatability of the seed (Oluwaniyi *et al.*, 2011).

Removing the toxic substance present in the seed has been attempted with satisfactory results by Oluwaniyi *et al.* (2011). The glucoside content was reduced by 95% when the seed was detoxified with acid followed by an alcoholic extraction of the glycoside. The direct alcohol detoxification of the seed meal led to a 98% reduction in the glycoside content. Furthermore, they found feeding alcohol detoxified *C. thevetia* seed meal to poultry produced comparable growth performance to the control diet at 50% replacement of soybean meal in the diet (Oluwaniyi *et al.*, 2011). Also, detoxification of the seed improved the amino acid profile, especially the percentages of essential, aromatic, sulphur and basic amino acids (Akinpelu and Amoo, 2017).

This study was initiated to evaluate the effects of *C. thevetia* seed meal as an alternative protein supplement in the diet of *C. gariepinus* fingerlings on growth performance, haematology and tissue proximate composition. To the best of our knowledge, the use of CTSM has not yet been reported in *C. gariepinus* and the findings will benefit the catfish aquaculture industry.

## MATERIALS AND METHODS

### Test ingredient collection and treatment

The *Cascabela thevetia* seeds used for this study were collected from *Cascabela thevetia* trees in Ikole Ekiti, Ikole Local Government Area, Ekiti State Nigeria. The seeds were sun-dried and the seed cotyledon was removed by breaking open the hard seed coats. Oil in the seed cotyledons was extracted with a manual oil extractor in the nutrition laboratory of the Department of Fisheries and Aquaculture Federal University, Oye-Ekiti, Nigeria. The residue after oil extraction was detoxified with the solution of ethanol: methanol (8:2) as reported previously by Oluwaniyi *et al.* (2007). The ethanol: methanol solution was used to soak the defatted meal twice. 1000 ml of the solvent was used to soak 100 g of the defatted meal. The mixture was stirred with a magnetic stirrer set at 200 rpm for 45 min and left to stand for 24 h. The solvent was then decanted, and fresh solvent was added (500 ml to 100 g). The mixture was stirred and allowed to stand for 72 h, the final product was pressed free of solvent and detoxified samples were air-dried (at ambient temperature) to remove residual solvent.

### Fish and feeding trial

The feeding trial was conducted for 8 weeks at the Nutrition Laboratory of Federal University, Oye-Ekiti, Nigeria. One hundred and fifty *Clarias gariepinus* fingerlings were obtained from the University hatchery. The mean weight of six weeks old fish was 6.22±0.97 g. On arrival, the fish were acclimatized for two weeks in fifteen 60 l plastic tanks. During acclimation, fish were fed to apparent satiation twice daily using a commercial catfish feed. After the acclimation period, 10 fishes were batch weighed and stocked in flow-through tanks. Three tanks were randomly allocated to the five experimental diets in a completely randomized design (CRD).

Water quality was maintained by continuous aeration and a flow rate of 0.8 l/min per tank. Dissolved oxygen and ammonia concentrations were within the normal range recommended by Viveen *et al.* (1985). Natural photoperiod of 12:12 light/dark cycle was observed during the feeding trial. Fish were fed 5% of their body weight twice daily (between 09:00 – 09:30 and 16:00 – 16:30) for 8 weeks.

Five isonitrogenous and isocaloric diets (Table 1) were formulated to contain either 0 g/kg, 50 g/kg, 100 g/kg, 150 g/kg or 200 g/kg *Cascabela thevetia* seed meal. The diets were designated as CTSM0, CTSM50, CTSM100, CTSM150 or CTSM200 respectively for the levels of *Cascabela thevetia* seed meal which replaced soybean meal in diet formulations. CTSM0 diet served as the control diet. The diets were made into a pellet with meat mincer through a 2 mm die, sundried, packed in a sealed polythene bag and stored at -10°C until used.

**Table 1:** Composition of the experimental diets and CTSM (g/kg)

Ingredient	CTSM0	CTSM50	CTSM100	CTSM150	CTSM200	CTSM
Fish meal	300.0	300.0	300.0	300.0	300.00	
Groundnut cake	140.0	140.0	140.0	140.0	140.00	
Maize	99.6	99.6	99.6	99.6	99.6	
Wheat offal	50.0	50.0	50.0	50.0	50.0	
<i>Cascabela thevetia</i> seed meal	0.0	50.0	100.0	150.0	200.0	
Soy-bean meal	330.1	280.1	230.1	180.1	130.1	
<sup>1</sup> Vitamin/mineral premix	20.0	20.0	20.0	20.0	20.0	
Vitamin C	0.3	0.3	0.3	0.3	0.3	
Methionine	10.0	10.0	10.0	10.0	10.0	
Cassava starch	20.0	20.0	20.0	20.0	20.0	
Vegetable oil	30.0	30.0	30.0	30.0	30.0	
<i>Proximate composition (n=3)</i>						
Moisture	81.9	76.1	79.1	82.6	80.0	80.4
Crude Protein	404.9	407.1	409.4	407.3	403.0	477.8
Lipid	202.5	180.8	175.6	180.4	179.4	45.6
<sup>2</sup> NDF	259.0	275.2	276.4	279.6	284.8	294.2
<sup>3</sup> ADF	79.3	92.0	92.8	95.4	99.4	106.8
Ash	55.2	56.2	57.2	58.1	61.2	63.4
<sup>4</sup> Metabolizable energy (kJ/g)	10.6	10.8	10.9	10.7	10.6	12.7

CTSM = *C. thevetia* seed meal, CTSM0 contain 0 g/kg *C. thevetia* seed meal, CTSM50 contain 50 g/kg *C. thevetia* seed meal, CTSM100 contain 100 g/kg *C. thevetia* seed meal, CTSM150 contain 150 g/kg *C. thevetia* seed meal, CTSM200 contain 200 g/kg *C. thevetia* seed meal

<sup>1</sup>Vitamin/mineral premix supplied the following (per kg of diet): calcium, 4500 mg; phosphorus, 4200 mg; potassium, 1700 mg; magnesium, 400 mg; iron, 30 mg; zinc, 30 mg; manganese, 20 mg; copper, 5 mg; iodine, 1 mg; selenium, 0.25 mg; vitamin A, 5000 IU; vitamin D, 2000 IU; DL- $\alpha$ -tocopherol acetate, 100 mg; menadione, 15 mg; thiamine hydrochloride, 5 mg; riboflavin, 10 mg; pyridoxine hydrochloride, 10 mg; pantothenic acid, 35 mg; nicotinic acid, 50 mg; biotin, 0.5 mg; folic acid, 2 mg; ascorbic acid, 200 mg; inositol, 250 mg; choline, 400 mg; vitamin, B<sub>12</sub>, 0.1 mg; and ethoxyquin, 60 mg.

<sup>2</sup>Neutral detergent fibre.

<sup>3</sup>Acid detergent fibre.

<sup>4</sup>Calculated based on physiological fuel value of 16.7, 16.7 and 37.7 kJ/g for carbohydrate, protein and lipid, respectively.

### Sample collection and analysis

The experimental diets and fish tissue samples were analyzed for proximate composition using standard methods (AOAC 2003). Moisture (934.01) was determined gravimetrically after drying in an oven at 105°C for 24 h, ash (942.05) by incineration in a muffle furnace at 450°C for 16 h, protein (CP; 954.01) (N x 6.25) by the Kjeldahl method, lipid (EE; 920.39) by petroleum extraction. The neutral detergent fibre (NDF) and acid detergent fiber (ADF) were analyzed following the methods of Van Soest *et al.* (1991).

Fish were sampled and weighed fortnightly. Final sampling was carried out at the end of the study, where fish were anaesthetized with a solution of methane sulphonate (MS222) (Wagner *et al.*, 1997). The caudal peduncle of 2 fish per tank was severed with razor blade and blood samples were collected from the caudal vein into bottles containing heparin as an anticoagulant. One blood sample per replicate – three per treatment – was used for the analysis of the haematological parameters. For Red and white blood cell counts, preparation of blood smears was done after sampling using standard haematological techniques (Dacie and Lewis, 2001). Packed cell volume (PCV) was determined with 50  $\mu$ l haematocrit tube and microhaematocrit centrifuge. The tube was filled with blood samples and centrifuged at 3500 x g for 10 min for each blood sample. The Wintrobe and Westergreen method as described by Blaxhall and Daisley (1973) was used. The method described by Wedemeyer and Yasutake (1977) was used for the determination of haemoglobin (Hb) concentration. The formulae of Dacie and Lewis (2001) was used for the determination of MCV, MCH and MCHC using the RBC, Hb and PCV concentrations:

$$\text{MCV (fl)} = \text{PCV/RBC} (10^6 \mu\text{l}^{-1})$$

$$\text{MCH (pg)} = [\text{Hb (gd}^{-1}) \times 10] / \text{RBC} (10^6 \mu\text{l}^{-1})$$

$$\text{MCHC (gl}^{-1}) = [\text{Hb (gd}^{-1}) \times 10] / \text{PCV}$$

Calculations of other parameters were carried out as follows:

$$\text{Specific growth rate} = [\ln (\text{final weight}) - \ln (\text{initial weight}) / \text{period (days)}] \times 100$$

$$\text{Feed conversion ratio} = \frac{\text{feedintake}}{\text{weight gained}}$$

### Statistical analysis

The data obtained were subjected to one-way analysis of variance (ANOVA) using Statistical analysis system (SAS) programs version 9.0 (Cary, NC, USA). When significant treatment effect was observed, Tukey's honestly significant difference (HSD) *post hoc* test was used to compare the differences between treatment means and were considered significant at  $P < 0.05$  level of significance.

## RESULTS

Survival of the fish was not significantly different in all treatments (Table 2). Weight gain of *C. gariepinus* fed *C. thevetia* seed meal diets ranged from 5.54 to 7.61 g. Fish fed diet CTSM50 had the least weight gain and was like those of fish fed CTSM0, CTSM100, CTSM150 and CTSM200 diets. Feed intake of *C. gariepinus* fingerlings fed the experimental diets was significantly different ( $P > 0.05$ ). Higher feed intake ( $8.39 \pm 0.76$ ) was recorded in fish fed CTSM100 and lowest ( $6.10 \pm 0.12$ ) in fish fed CTSM50. The feed conversion ratio of fish fed CTSM150 ( $1.80 \pm 0.39$ ) was the highest and was not significantly different ( $P > 0.05$ ) from that of *C. gariepinus* fingerlings fed Control diet, CTSM50, CTSM100 and CTSM200 diets. Similarly, the protein efficiency ratio was not significantly different ( $P > 0.05$ ) in *C. gariepinus* fingerlings fed the experimental diets. Specific growth rate (SGR) of *C. gariepinus* fingerlings were similar ( $P > 0.05$ ) in all experimental diets. Highest SGR ( $1.67 \pm 0.12$ ) were observed in fish fed CTSM200 and lowest ( $1.13 \pm 0.25$ ) in fish fed CTSM50 diet. Similarly, the relative growth rate of *C. gariepinus* fingerlings fed the experimental diets were not significantly different.

The proximate tissue composition of the fish fed the experimental diet is presented in Table 3. Moisture content was highest in diet CTSM200 ( $709.60 \pm 0.26$ ), while diet CTSM50 had the lowest moisture content. Diet CTSM0 (control) had the highest value for tissue protein ( $197.70 \pm 2.35$ ), this was similar ( $P > 0.05$ ) to the tissue protein of fish fed diet CTSM200 ( $190.03 \pm 4.42$ ) and were significantly different ( $P < 0.05$ ) from other dietary treatments. Fish fed diet CTSM50 had the lowest tissue protein content ( $160.60 \pm 2.90$ ). Tissue lipid of *C. gariepinus* fed control diet was significantly ( $P < 0.05$ ) higher than that of fish in other dietary groups.

Table 4 shows the Haematological parameters of *C. gariepinus* fingerlings fed *C. thevetia* seed meal diets. Packed cell volumes of fish fed CTSM0, CTSM100 and CTSM150 were not significantly different ( $P > 0.05$ ) but were significantly different from fish fed CTSM200. Haemoglobin of *C. gariepinus* fed Control diet (CTSM0), CTSM50, CTSM100, and CTSM150 were not significantly different ( $P > 0.05$ ) but were significantly different ( $P < 0.05$ ) from fish fed CTSM200, with lowest haemoglobin in fish fed control diet ( $10.89 \pm 0.69$ ) and highest in CTSM200 ( $14.11 \pm 0.51$ ) fed fish. The red blood cell counts (RBC) of fish fed the experimental diet shows that the red blood of fish fed the control diet was similar ( $P > 0.05$ ) to that of fish fed CTSM50 diet but was significantly different ( $P < 0.05$ ) from the red blood cell of fish fed CTSM100, CTSM150 and CTSM200.

Mean corpuscular haemoglobin and mean corpuscular volume (MCV) of fish fed CTSM200 diet was significantly different ( $P < 0.05$ ) from CTSM0, CTSM50, CTSM100 and CTSM150 fed fish. Similarly, significant differences ( $P < 0.05$ ) were observed in the results of Mean corpuscular haemoglobin concentration of the fish fed the experimental diets. White blood cell counts in fish fed the control diet, CTSM50, and CTSM100, were similar but significantly different ( $P < 0.05$ ) from those of CTSM200 fed fish. The values obtained for neutrophil and lymphocytes showed that there were no significant differences among the dietary groups.

**Table 2:** Growth performance of *Clarias gariepinus* fingerlings fed diets containing varying levels of detoxified *Cascabela thevetia* seed meal fed for eight weeks

Parameter	CTSM0	CTSM50	CTSM100	CTSM150	CTSM200	pSEM	P-value	F-value
Initial weight (g)	5.9 <sup>a</sup>	6.3 <sup>a</sup>	6.0 <sup>a</sup>	6.4 <sup>a</sup>	6.0 <sup>a</sup>	0.968	0.5120	0.88
Final weight (g)	12.1 <sup>a</sup>	11.8 <sup>a</sup>	12.5 <sup>a</sup>	12.9 <sup>a</sup>	13.2 <sup>a</sup>	0.821	0.3176	1.35
<sup>1</sup> Weight gain (g)	6.2 <sup>a</sup>	5.5 <sup>a</sup>	6.5 <sup>a</sup>	6.6 <sup>a</sup>	7.6 <sup>a</sup>	1.122	0.2044	1.81
Feed intake (g)	7.0 <sup>ab</sup>	6.1 <sup>b</sup>	8.4 <sup>a</sup>	8.3 <sup>a</sup>	8.1 <sup>a</sup>	0.853	0.0292	4.24
<sup>2</sup> FCR	1.1 <sup>a</sup>	1.1 <sup>a</sup>	1.5 <sup>a</sup>	1.3 <sup>a</sup>	1.1 <sup>a</sup>	0.253	0.2094	1.78
<sup>3</sup> PER	0.2 <sup>a</sup>	0.1 <sup>a</sup>	0.2 <sup>a</sup>	0.2 <sup>a</sup>	0.2 <sup>a</sup>	0.030	0.1043	2.56
<sup>4</sup> SGR (%)	1.3 <sup>a</sup>	1.1 <sup>a</sup>	1.3 <sup>a</sup>	1.3 <sup>a</sup>	1.4 <sup>a</sup>	0.281	0.2946	1.43
<sup>5</sup> Survival (%)	93.33 <sup>a</sup>	93.33 <sup>a</sup>	90.00 <sup>a</sup>	96.67 <sup>a</sup>	93.33 <sup>a</sup>	6.831	0.8335	0.36

CTSM0 contain 0 g/kg *C. thevetia* seed meal, CTSM50 contain 50 g/kg *C. thevetia* seed meal, CTSM100 contain 100 g/kg *C. thevetia* seed meal, CTSM150 contain 150 g/kg *C. thevetia* seed meal, CTSM200 contain 200 g/kg *C. thevetia* seed meal. Values are means from triplicate groups of fish, where the means in each row with the same superscripts are not significantly different ( $P > 0.05$ ).

<sup>1</sup>Weight gain = final weight – initial weight.

<sup>2</sup>FCR - Feed conversion ratio = dry feed intake/wet weight gain.

<sup>3</sup>PER - Protein efficiency ratio = wet weight gain/protein intake.

<sup>4</sup>SGR - Specific growth rate =  $(\log_e \text{ final weight} - \log_e \text{ initial weight}) \times 100/\text{days}$ .

<sup>5</sup>Survival =  $(\text{total fish} - \text{dead fish}) \times 100/\text{total fish}$ .

**Table 3:** Tissue proximate composition of *Clarias gariepinus* fed diets containing *Cascabela thevetia* seed meal fed for eight weeks (g/kg)

Parameters	CTSM0	CTSM50	CTSM100	CTSM150	CTSM200	SEM	P-value	F-value
Moisture	706.0 <sup>b</sup>	701.0 <sup>a</sup>	701.9 <sup>a</sup>	705.5 <sup>b</sup>	709.6 <sup>c</sup>	0.084	<0.0001	50.51
Protein (%)	197.7 <sup>c</sup>	160.6 <sup>a</sup>	170.7 <sup>b</sup>	176.3 <sup>b</sup>	190.0 <sup>c</sup>	0.344	<0.0001	55.86
Lipid (%)	13.1 <sup>b</sup>	8.6 <sup>a</sup>	9.2 <sup>a</sup>	8.9 <sup>a</sup>	9.2 <sup>a</sup>	0.027	<0.0001	147.49
Ash (%)	35.7 <sup>a</sup>	38.6 <sup>b</sup>	40.2 <sup>c</sup>	50.1 <sup>d</sup>	50.4 <sup>d</sup>	0.041	<0.0001	846.64

CTSM0 contain 0 g/kg *C. thevetia* seed meal, CTSM50 contain 50 g/kg *C. thevetia* seed meal, CTSM100 contain 100 g/kg *C. thevetia* seed meal, CTSM150 contain 150 g/kg *C. thevetia* seed meal, CTSM200 contain 200 g/kg *C. thevetia* seed meal. Values are means from triplicate groups of fish, where the means in each row with different superscripts are significantly different ( $P < 0.05$ )

**Table 4:** Haematological profile of *Clarias gariepinus* fingerlings fed diets containing *Cascabela thevetia* seed meal fed for eight weeks

Parameter	CTSM0	CTSM50	CTSM100	CTSM150	CTSM200	pSEM	P-value	F-value
<sup>1</sup> PCV (%)	34.7 <sup>ab</sup>	41.7 <sup>bc</sup>	34.0 <sup>ab</sup>	33.3 <sup>a</sup>	47.3 <sup>c</sup>	2.875	0.0005	13.54
<sup>2</sup> Hb (gdl <sup>-1</sup> )	10.9 <sup>a</sup>	12.9 <sup>ab</sup>	11.3 <sup>a</sup>	11.1 <sup>a</sup>	14.1 <sup>b</sup>	0.959	0.0086	6.28
<sup>3</sup> RBC (x10 <sup>6</sup> μl <sup>-1</sup> )	3.47 <sup>c</sup>	3.17 <sup>bc</sup>	2.40 <sup>ab</sup>	2.30 <sup>a</sup>	2.73 <sup>a</sup>	0.300	0.0002	16.27
<sup>4</sup> MCV (fl)	100.0 <sup>a</sup>	131.7 <sup>b</sup>	141.9 <sup>b</sup>	146.7 <sup>b</sup>	274.9 <sup>c</sup>	11.402	<0.0001	104.38
<sup>5</sup> MCH (pg)	54.4 <sup>a</sup>	64.5 <sup>ab</sup>	56.7 <sup>a</sup>	55.6 <sup>a</sup>	70.6 <sup>b</sup>	4.795	0.0086	6.28
<sup>6</sup> MCHC (g l <sup>-1</sup> )	31.4 <sup>c</sup>	30.9 <sup>b</sup>	33.3 <sup>d</sup>	33.3 <sup>d</sup>	29.8 <sup>a</sup>	0.107	<0.0001	625.44
<sup>7</sup> WBC (x10 <sup>9</sup> μl <sup>-1</sup> )	283.3 <sup>a</sup>	323.3 <sup>ab</sup>	413.3 <sup>ab</sup>	526.7 <sup>bc</sup>	683.7 <sup>c</sup>	77.028	0.0005	13.34
Neutrophil (%)	18.0 <sup>a</sup>	28.3 <sup>a</sup>	32.3 <sup>a</sup>	30.7 <sup>a</sup>	23.0 <sup>a</sup>	9.726	0.4066	1.10
Lymphocyte (%)	82.0 <sup>a</sup>	71.7 <sup>a</sup>	67.7 <sup>a</sup>	69.3 <sup>a</sup>	77.0 <sup>a</sup>	9.726	0.4066	1.10

CTSM0 contain 0 g/kg *C. thevetia* seed meal, CTSM50 contain 50 g/kg *C. thevetia* seed meal, CTSM100 contain 100 g/kg *C. thevetia* seed meal, CTSM150 contain 150 g/kg *C. thevetia* seed meal, CTSM200 contain 200 g/kg *C. thevetia* seed meal. Values are means from triplicate groups of fish, where the means in each row with different superscripts are significantly different ( $P < 0.05$ ).

<sup>1</sup>Packed cell volume.

<sup>2</sup>Haemoglobin

<sup>3</sup>Red blood cell.

<sup>4</sup>Mean corpuscular cell volume.

<sup>5</sup>Mean corpuscular haemoglobin

<sup>6</sup>Mean corpuscular haemoglobin concentration

<sup>7</sup>White blood cell.

## DISCUSSION

In the present study, the growth performance of fish improved with the replacement of soybean meal with detoxified *C. thevetia* seed meal. As a result of the treatment, weight gain, feed intake, protein efficiency ratio and specific growth rate of fish fed *C. thevetia* seed meal-based diets increased. This indicates that the test ingredient's toxic component has been removed or reduced to an insignificant level, leading to improved digestibility, absorption, and utilization of the nutrients. The performance of fish fed CTSM200 was higher, although not significantly different from fish in other dietary treatments. This result is comparable with the results of Oluwaniyi *et al.* (2011) who reported improved growth performance when 15% of *C. thevetia* seed meal was incorporated in the diet of cockerel but Taiwo *et al.* (2004) recommended 5% inclusion of the seed meal majorly due to the mode of treatment method used. This study's higher inclusion level (20%) shows that fish can utilize detoxified CTSM with improved growth performance than a cockerel.

Crude protein of the fish after the experimental period has an average of 19.00%, which was like the report of Adebayo *et al.* (2016), Fagbenro *et al.* (2010) and differ from the report of Oladipo and Bankole (2013) who reported 17.50%, the differences recorded may be due to the ability of the fish to metabolize and utilize essential nutrients from the diets and the nature and quality of nutrients present in the experimental diets (Adebayo *et al.*, 2016).

Fish haematology is essential in monitoring the health status of fish (Ahmed and Sheikh 2020). Significant differences were recorded in the haematological parameters of *C. gariepinus* fed the *C. thevetia* seed meal diets in this study. It has been shown that the haematology of any animal, catfish inclusive is traceable to the diet fed to the fish, or its environment, which could be due to stress or bad water quality parameters (Arejinuwa *et al.*, 2002). This is important in monitoring feed toxicity, especially with feed constituents that affect the formation of blood in culture fisheries (Falaye, *et al.*, 2018). High value of PCV, Hb and WBC recorded in fish fed CTSM200 compared to the other diets implies that the addition of CTSM to the diets of *C. gariepinus* had no negative effects on the blood parameters. Reduction in PCV concentrations in fish blood usually suggests the presence of toxic factors like haemagglutinin which adversely affects blood formation (Tiamiyu *et al.*, 2019). This study shows a reduction or absence

of the toxic element in the infeed; hence, signs of stress were not pronounced in the fish and the fish were not anaemic as revealed by the PCV values recorded in the study. Low Hb may impair oxygen supply to various tissues, leading to slow metabolic rate and low energy production. This suggests a predisposition to anaemia. Increased Hb values observed in this study indicate a higher rate of oxygen transport to and removal of carbon (IV) oxide from the body tissues of *C. gariepinus*. This results in higher metabolism and growth. Compared with the results of the previous study from different freshwater fishes, the blood parameter values obtained in the present study were higher than the values obtained in *C. gariepinus* (Ochang *et al.*, 2007), *Heterobranchus longifilis* (Babalola *et al.*, 2009, 2016) and *Oreochromis niloticus* (Iheanacho *et al.*, 2018).

Important tools in the diagnosis of anaemia in most domesticated animals are the blood indices (MCV, MCH and MCHC). A significant decrease in MCV and MCH has observed in fish fed the control diet in this study. This suggests a possible hemoconcentration as observed in *Prochilodus scrofa* exposed to copper (Mazon *et al.*, 2002) and *L. rohita* exposed to effluents of paint, dye and petroleum industry (Zutshi *et al.*, 2010). Similarly, low MCHC values indicate hypochromic anaemia, as observed by Javed *et al.* (2016).

### CONCLUSION

It is imperative to search for alternative protein sources in the fish diet. The conventional protein feedstuffs are scarce and expensive and most of the fish farmers cannot afford them in fish diets. This study has clearly shown that *C. thevetia* seed meal can effectively replace soybean meal in fish diets is evident in the similar growth performance of fish fed CTSM based diets and the control that contains soybean meal. Similarly, the haematological profile of the fish was not negatively affected with the increased dietary inclusion of *C. thevetia* seed meal. Therefore, minimal stress is placed on the health of *Clarias gariepinus* fingerlings even when fed at a higher inclusion level (200 g/kg).

### CONFLICT OF INTEREST:

The authors declare no conflict of interest

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