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Research article

Impact on Growth and Nutrient Utilization on Health and Hematological Effects of *Cassia tora* (L) on *Clarias gariepinus* (Burchell, 1882)

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Abstract
The African catfish *(Clarias gariepinus*) is the most cultivated species with desirable characteristics. The hematological parameters change in *C. gariepinus* fed varying inclusion of *Cassia tora* (leaf and seeds) meals. The aim of this study was to determine the impact on growth and nutrient utilization on health and hematological effects of *Cassia tora* (L) on C*larias gariepinus* (Burchell, 1882). *C. tora* leaves and seeds were washed, shed dried, and ground with mortar and pestle into powder. The ingredients were ground into powder, weighed, and mixed thoroughly with the addition of warm water to make a dough and pellet using form pellet. *Clarias gariepinus* juveniles were acclimatized to experimental conditions for 7 days prior to the feeding trials. A group of catfish with an average body weight of 4.3 g and standard length of 6.41 cm were stocked in 15 aquariums comprising 20L capacity circular tanks. The results showed that there is a significant difference in RBC, with the seed meal having the highest values. There were no significant differences in WBC. The PCV was within the minimum required standard; there was significance in MCH, MCV, HB, and MCHC. The highest values of HB and MCHC were obtained in *Clarias gariepinus* fed leaf and seed meals. However, there was mild stress placed on the health of the catfish even when fed at high percentage levels of inclusion (100%). Therefore, the inclusion level of (25%) of *C. tora* (leaf and seed meals) was recommended in the feed formulation using *C. tora***.**

Keywords: *Clarias gariepinus,* Heamatology, *C. tora,* Carcass composition, Full blood count.

INTRODUCTION

Clarias gariepinus is a member of the family *Clariidae*. In Nigeria, fish production has attracted significant attention compared to other animal-producing sectors in terms of growth (Udo and Umanah, 2017). Nigeria is considered one of the highest fish-consuming nations in Africa, with relatively high per capita consumption levels (Chan *et al*., 2019; Adeleke *et al.,* 2020). The quest for alternative sources of animal feed that do not compete with human food sources has been the focus of several cost-oriented studies (Esonu *et al*., 2004; Ranza-Faival *et al.,* 2000; Udedibie *et al*., 2005; Emenalom *et al.,* 2009). One promising alternative is the leaf and seed of the sickle pod (*C. tora),* which has significant potential as a cost-effective energy source in monogastric diets, particularly for poultry. It is readily available and does not compete with human food or industrial uses (Assam *et al.*, 2017; Gabriel *et al.*, 2001).

Hematology is a branch of medicine that studies blood, the organs that produce it (Fazio *et al*., 2017), and the diseases that affect it. The term "heme" comes from the Greek word for blood (Fagbenro *et al.*, 2013). Hematology is practiced by experts in the field who handle the diagnosis, treatment, and overall management of individuals with blood disorders ranging from anemia to blood cancers. Some of the conditions treated by hematologists include iron deficiency anemia, other types of anemia such as sickle cell anemia, polycythemia (excess production of red blood cells), *myelofibrosis, leukemia*, and platelet and bleeding disorders (Fischbech, 1992; Anthony *et al.,* 2010).

In fish physiology, hematological methods are frequently used to assess their physiological state and health (Martins *et al*., 2011; Fazio, 2019). Fish hematological research dates back to 1943 (Bianchi *et al.*, 2014). Since then, there has been significant advancement in the literature on various methods of fish blood cell analysis, and our understanding of fish blood analysis has grown substantially (Stoskopf, 1993; Fazio *et al*., 2017; Lowrence *et al*., 2020). Henceforth, these studies investigate the haematological characteristics of *C. gariepinus* fed *Cassia tora* (leaf and seeds) dietary supplement based diets.

MATERIALS AND METHODS

Experimental Site

The research work was conducted at the Biological Sciences Laboratory (11°45’22N, 9°20’20E), Department of Biological Sciences, Federal University Dutse, Jigawa State, Nigeria.

Experimental Design

A ten-week feeding trial was conducted on *Clarias gariepinus* using *Cassia tora* (leaf and seed). The leaves and seeds were collected from the Botanical Garden, Federal University, Dutse, Nigeria. The *C. tora* (leaf and seed) of the plant were taken to the Department of Plant Biology, Herbarium Bayero University, Kano, and identified (Herbarium Accession Number BUKHAN 0307). The leaves were washed, shed dried, and ground with a mortar and pestle into powder. The seeds were manually threshed from their pods and washed. The seeds were then boiled at 100°C for 40 minutes. The air-dried and boiled seeds were weighed and oven-dried at 80°C in a paper bag for 24 hours, followed by cooling in a desiccator for dry matter determination. The dried seeds were pulverized using a laboratory blender and sieved using a 0.5 mm mesh sieve (Ingweye *et al.*, 2010b). The flour was stored in screw-capped bottles at room temperature for further analyses. Part of the *Cassia tora* (leaves and seed) powder was taken to the Institute for Agricultural Research, Ahmadu Bello University, Zaria, for proximate analysis. The ingredients were ground into powder (Table 1), weighed, and mixed thoroughly with the addition of warm water to make a dough and pellet using form pellet.

Fish Management and Experimental Diets

Clarias gariepinus juveniles were acclimatized to experimental condition for 7 days prior to the feeding trails. A group of 10 catfish juveniles with average body weight of 4.3g and standard length of 6.41cm were stocked in 15 aquarium comprising 20L capacity circular plastic tanks. The fish fed morning and evening at 5% of their body weight, the inclusion levels were 0% (controls), 25%, 50%, 75% and 100%. The weighting was done using digital weighing balance (CAMRY EK5350).

Table 1. Percentage Composition of Various Inclusion Levels of *Cassia tora* in the Experimental Feeds.

Ingredients	(0%)	(25%)	(50%)	(75%)	(100%)
Fish meal	25.02	18.84	12.66	6.48	-
Soya bean	23.51	23.51	23.51	23.51	23.81
Maize	22.75	22.75	22.75	22.75	22.75
Cassia tora	-	6.18	12.36	18.54	24.72
Ground nut	24.72	24.72	24.72	24.72	24.72
Bone meal	01	01	01	01	01
Starch	0.50	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50	0.50
Vegetable oil	01	01	01	01	01
Vit. Premix	1	1	1	1	1
Total	100	100	100	100	100

Full Blood Count
The full blood count was determined according to the methods conducted by Svobodova *et al.* (1991) as follow:

Blood analysis
A number 5-10mL blood samples were collected from the cardiac puncture using 2mL disposal heparinised syringe treated with EDTA as anti-coagulant.

Blood cell count
Heamocytometer was used in blood cell count. The blood diluting fluid was prepared as described by Svobodova *et al* (1991). The blood cells was counted on the counting chamber of haemocytometer with the aid of compound microscope:

RBC = No. of cells count ×3×10×200 (10^6 mm^3)
WBC = No. of cells count ×0×25×20 (10^4 mm^3)

Haemoglobin estimation: Heamoglobinometer was used for haemoglobin estimation based on acid haematin method (SAHLI):

$$Haemoglobin = \frac{\text{Value obtained}}{100} \times 17.2mg/100mL$$

Packed cell volume:
The packed cell volume was measured by placing a sealed micro-haematocrit tube in a centrifuge at 10,500 rpm, using a micro-haematocrit reader, and expressed as a percentage.

Erythrocytes sedimentation rate (ESR)
ESR was determined following the procedure outlined by Svobodova *et al*. (1991). It represents the difference between 100% and the percentage of the corpuscle volume within the specified time interval.

Mean corpuscular volume (MCV): MCV was calculated from the heamatocrit value (PCV, % and the erythrocytes count (Er mm^3).

$$MCV(\mu^3) = \frac{PCV}{Er} \times 10$$

Mean corpuscular haemoglobin concentration (MCHC): This was obtained using the formula:

$$MCHC(\%) = \frac{Hb}{PCV} \times 100$$

Mean corpuscular haemoglobin (MCH): This was expressed either in a table or pictograms (pg):

$$MCH(\text{pg}) = \frac{Hb}{Er} \times 10^2$$

Data Analysis
The data were subjected to two-way analysis of variance (ANOVA) to test for significant differences in the means. When means were significantly different, Duncan's multiple range test was used to separate them. Analysis was performed using the Data Tab (Online Statistical Package of Sciences).

Table 2. Percentage Composition of *C. tora*

Parameters	Dry Matter	Crude Protein	Crude Fibre	Either Extracts	Ash	Nitrogen Free Extracts
Leaf	92.98 ± 1.94^a	24.22 ± 3.15^a	19.94 ± 1.21^c	12.41 ± 0.42^c	12.23 ± 0.23^a	37.9 ± 0.57^a
Seeds	94.28 ± 1.2^a	23.6 ± 1.54^a	19.71 ± 0.79^b	19.42 ± 0.28^a	12.61 ± 0.3^d	38.28 ± 0.9^d
Leaves and seeds	97.86 ± 1.2^a	26.24 ± 1.27^a	20.13 ± 0.46^c	15.05 ± 0.15^d	12.08 ± 0.33^a	39.09 ± 0.24^b

Means with different superscript along same column are significantly different ($p \leq 0.05$).

Table 3. Carcass Composition of *Clarias gariepinus* Fed Experimental Feeds

Feeds	Moisture (%)	Ash (%)	Lipids (%)	Proteins (%)	Carbohydrates (%)
Leaves 0%	22.37 ± 5.55[a]	1.48 ± 0.33[a]	12.39 ± 4.03[a]	30.64 ± 16.58[b]	29.22 ± 3.07[d]
Leaves 25%	24.01 ± 2.58[a]	1.4 ± 0.09[a]	13.71 ± 4.62[a]	35.99 ± 6.49[b]	25.28 ± 3.76d
Leaves 50%	22.35 ± 2.05[a]	1.36 ± 0.2[a]	10.1 ± 0.76[a]	32.05 ± 2.31[b]	19.51 ± 19.06[d]
Leaves 75%	25.8 ± 2.01[a]	1.54 ± 0.4[a]	10.22 ± 1.88[a]	27.12 ± 5.26[d]	14.42 ± 12.14[b]
Leaves 100%	21.86 ± 0.68[a]	1.51 ± 0.34[a]	11.16 ± 2.32[a]	30.24 ± 3.33[b]	16.11 ± 15.12[b]
Seeds 0%	23.56 ± 1.78[a]	1.64 ± 0.27[a]	11.68 ± 3.43[a]	41.69 ± 7.96[d]	31.18 ± 1.82[b]
Seeds 25%	23.4 ± 1.75[a]	1.45 ± 0.35[a]	11.31 ± 1.65[a]	29.02 ± 2.83[b]	32.81 ± 2.63[d]
Seeds 50%	23.95 ± 2.87[a]	1.42 ± 0.31[a]	12.33 ± 1.28[a]	27.5 ± 2.87[d]	29.79 ± 5.22[b]
Seeds 75%	22.54 ± 1.07[a]	1.53 ± 0.34[a]	12.62 ± 2.9[a]	29.72 ± 3.04[d]	12.39 ± 2.8[d]
Seeds 100%	22.7 ± 1.08[a]	1.35 ± 0.32[a]	12.46 ± 3.36[a]	26.96 ± 4.21[d]	19.6 ± 7.23[b]
Leaves and seeds 0%	23.52 ± 2.04[a]	1.46 ± 0.13[a]	11.53 ± 2.1[a]	39.88 ± 3.99[b]	34.54 ± 6.77[d]
Leaves and seeds 25%	22.31 ± 0.86[a]	1.58 ± 0.27[a]	11.71 ± 2.09[a]	50.45 ± 2.2[b]	31.95 ± 1.46[d]
Leaves and seeds 50%	22.76 ± 0.6[a]	1.44 ± 0.39[a]	11.86 ± 2.2[a]	28.51 ± 3.42[d]	31.43 ± 3.24[b]
Leaves and seeds 75%	22.36 ± 0.54[a]	1.66 ± 0.3[a]	11.79 ± 0.96[a]	31.8 ± 2.67[b]	33.59 ± 4.98[d]
Leaves and seeds100%	22.07 ± 0.61[a]	1.58 ± 0.23[a]	10.19 ± 3.73[a]	32.28 ± 3.1[b]	14.88 ± 6.53[b]

Mean with different superscript along same column are significantly different ($p \leq 0.05$)

Table 4: Hematological Parameters of *Clarias gariepinus* Fed *Cassia tora* Feeds.

Feeds	RBC (×106L-1)	WBC (×103)	MCV (μ3)	MCHC (%)	MCH (pg)	PCV (%)	HB (gdl-1)
Leaves 0%	2.11 ± 0.99*	2.2 ± 1.58*	9.2 ± 0.11*	13.5 ± 0.12*	13.72 ± 0.75*	23 ± 0.25*	3.32 ± 0.18*
Leaves 25%	3.04 ± 0. 91*	2.2 ± 1.1*	9.28 ± 0.59*	14.3 ± 1.44*	14.86 ± 1.25*	23.2 ± 1.48*	3.44 ± 0.13*
Leaves 50%	3.06 ± 0.6*	1.16 ± 0.79*	9.36 ± 0.4*	14.24 ± 1.48*	14.94 ± 1.38*	23.4 ± 1.14*	3.48 ± 0.16*
Leaves 75%	3.03 ± 0.6*	2.12 ± 0.76*	9.92 ± 0.18*	14.36 ± 0.88*	14.68 ± 0.59*	24.8 ± 0.45*	3.64 ± 0.17*
Leaves 100%	3.12 ± 0.9*	3.04 ± 0.78*	9.84 ± 0.46*	15.94 ± 0.92*	15.94 ± 0.92*	24.6 ± 1.14*	3.92 ± 0.28*
Seeds 0%	3.4 ± 3.6*	1.9 ± 1.3*	9.68 ± 0.52*	13.34 ± 1.05*	14.96 ± 1.07*	24.2 ± 1.3*	3.28 ± 0.16*
Seeds 25%	3.12 ± 0.78*	1.3 ± 0.79*	8.96 ± 0.67*	14.96 ± 1.07*	15.3 ± 1.98*	22.4 ± 1.67*	3.34 ± 0.11*
Seeds 50%	3.5 ± 1.26*	2.8 ± 0.79*	9.68 ± 0.33*	15.32 ± 1.04*	15.28 ± 0.78*	24.2 ± 0.84*	3.7 ± 0.21*
Seeds 75%	3.04 ± 0.91*	2.52 ± 0.48*	9.84 ± 0.46*	15.44 ± 0.48*	15.56 ± 1.1*	24.6 ± 1.14*	3.76 ± 0.25*
Seeds 100%	3.11 ± 1.24*	2.52 ± 0.64*	10 ± 0.28**	15.68 ± 0.44*	14 ± 1.71*	25 ± 0.71*	3.92 ± 0.28*
Leaves and seeds 0%	3.13 ± 0.8*	2.4 ± 0.93*	9.68 ± 1.04*	14.42 ± 2.05*	15.38 ± 1.56*	24.2 ± 2.59*	3.36 ± 0.15*
Leaves and seeds 25%	3.05 ± 0.91*	2.8 ± 0.84*	9.04 ± 0.61*	15.36 ± 1.57*	13.2 ± 1.11*	22.6 ± 1.52*	3.46 ± 0.18*
Leaves and seeds 50%	3.12 ± 0.79*	2.4 ± 1.14*	9.76 ± 0.54*	15.2 ± 1.11*	15.02 ± 0.67*	24.4 ± 1.34*	3.7 ± 0.12*
Leaves and seeds 75%	3.02 ± 1.24*	2.64 ± 1.16*	10 ± 0.28*	15.04 ± 0.65*	13.02 ± 0.67*	25 ± 0.71*	3.76 ± 0.23*
Leaves and seeds 100%	3.97 ± 0.87*	2.8 ± 0.45*	9.38 ± 1.08*	18.26 ± 2.53*	18.2 ± 1.78*	23.4 ± 2.7*	4.28 ± 0.15*

Mean with different superscript along same column are significantly different ($p \leq 0.05$)

Results and Discussion

The proximate composition of *C. tora* (Table 2) showed that there was no significant difference ($p \leq 0.05$) in DM, CF, EE, and Ash among the experimental feeds, but there was a significant difference in CP and NFE. This could be due to the heterogeneous nature of some of the samples (leaf and seed) and the method of processing. This is in agreement with the findings of Robinson *et al*. (2001). The crude protein level in diets may exceed 40%, while maintenance may contain as little as 25-35%. The moisture content ranged from 6.7% to 8.4% in feeds, which was not high. This clarified that the feed was properly dried to prevent fungal growth. According to Robinson *et al*. (2001), the typical way to deal with forming feed for basic stomach creatures is to use ingredients that will maintain dietary fiber levels. These levels would be in the range of 3-6% crude fiber for catfish feed this is in agreement with the finding of Umar *et al*. (2017).

However, there are high levels of crude protein and nitrogen-free extract, which signify that the crude protein of different feeds was isonitrogenous. This is greater for the study because the essential part of fish feed is the protein. All the trials were accepted by the fish, showing that the inclusion of *C. tora* in the feeds significantly affected the palatability of the feed. The reason could be the processing techniques used, which made the feeds palatable. This is consistent with the findings of Kwari *et al*. (2019), who reported that a decrease in anti-nutrient content by various processing methods resulted in better palatability, growth, and development in fish.

Table 3 showed carcass composition of *C. gariepinus* fed experimental feeds study showed an increase in protein among the treatments, with an average increase in carbohydrates and a significant decrease in moisture across the treatments. This could be due to the high protein content of *C. tora*, which is consistent with findings of Amerah *et al*. (2014) and Assam *et al*. (2017). The carcass crude protein of fish fed different dietary levels in this study was higher than that observed by Bake *et al.* (2016) for *C. gariepinus* fed different dietary levels of toasted *S. obtusifolia* seed meal. This confirms that boiled *S. obtusifolia* seed resulted in improved growth outcomes for *C. gariepinus* juveniles compared to toasted seed fed to *C. gariepinus* fingerlings as used in the study by Bake *et al.* (2016), likely due to the presence of anti-nutrients in the seed and feed utilization of fish at the fingerling stage.

In this study, a normal increase was observed (Table, 4) in the values of haematological parameters when the fish were fed experimental diets. The PCV values of 22% to 25% observed in this study fall within the range of 20% to 50% reported by Ibidunni *et al*. (2018) contrasting with the findings of Adesina *et al.* (2017) and Erondu *et al.* (1993). Similarly, the findings were lower than those reported by Adedeji and Adegbile (2011), Dienye and Olumiji (2014), and Mamman *et al.* (2013) when *C. gariepinus* was fed M. *oleifera* leaf meal and calabash seed cake individually. However, the study specified that *C. gariepinus* fed *Cassia tora* feeds showed a significant ($p < 0.05$) increase in haematocrit (PCV), haemoglobin, RBC, and WBC. This was consistent with the work of Haghigi and Rohani (2013), who reported a similar increase in haematological parameters in rainbow *trout* fed ginger powder. These findings were also consistent with Farah et al. (2012), who observed significant improvements (higher values) in WBC and PCV in diets supplemented with *M. officinalis* and *aloe vera*.

The Red Blood Cell (RBC) count was highest in fish fed the leaves and seed diet (100%) of *Cassia tora*, higher than in the other dietary treatments as well as the control diet. This could be due to the combined presence of anti-nutrients in both the leaf and seed separately. This observation agrees with Piotr *et al*. (2014), who affirmed that an increase in PCV can result from an increase in RBC count due to acute stress, spleen evacuation, erythrocyte swelling, or lower pH.

The haemoglobin concentration in this study was high, with no significant difference ($p \leq 0.05$) among the treatments. This could be attributed to decreased oxygen concentration in the experimental tanks, consistent with the findings of Kori-Siakpere and Ubogu (2008), who reported conflicting oxygen values in experimental situations such as 15.31 mg/l for juvenile hybrids and 9.63 g/dL × 103 for *C. gariepinus* (Ogunji *et al.*, 2005). Other oxygen values reported include 13.00 mg/l (Sunomonu and Oyelola, 2008), 18.43 and 16.00 g/dL × 103 (Onyia, 2013), and 6.80-6.90 g/100 ml for *C. gariepinus* fed sunflower seed meal (Adesina *et al.*, 2017).

However, in WBC count, there were normal increases among all treatments, with no significant difference ($p \leq 0.05$), likely due to the composition and percentage of the diet. This finding is similar to that of Ajani (2006) and Kori-Siakpere *et al.* (2009), who observed that a high WBC count indicates the release of more cells to maintain homeostasis, while a low WBC count is a common stress response. Increasing or decreasing numbers of WBCs are normal physiological reactions to toxicants, reflecting the response of the immune system under toxic conditions. Douglas and Jane (2010) suggest that WBCs play a crucial role in immune responses and the organism's ability to combat infections.

Additionally, the Mean Corpuscular Volume (MCV) showed no significant variation, suggesting that MCV was not affected by dietary treatments. MCV indicates the size or status of the RBCs and reflects normal or abnormal cell division during erythropoiesis. The values were lower than the 20.82 to 26.60 pg/dl recorded by Anyanwu *et al.* (2011) for *Heteroclarias* fed *Carica papaya* leaf meal and the 24.24 pg/dl for *C. gariepinus* juveniles (Omitoyin, 2007), but similar to the findings of Bamidele *et al.* (2015), who reported 15-20 pg/dl for *Clarias gariepinus* fed *Moringa oleifera* leaf seed meal. Mean Corpuscular Hemoglobin Concentration (MCHC) is a significant indicator of red blood cell density (Wepener *et al.*, 1992) and provides a quantitative measure of mean hemoglobin content per erythrocyte in organisms (Moses, 2007).

CONCLUSION

The percentage composition of *C. tora* obtained possessed the maximum amount of feed contents required for inclusion in aqua-feed, the haematological parameters of *Clarias gariepinus* was considered not significance ($p \leq 0.05$), the carcass composition showed better utilization of feeds with changes in the increased dietary percentage inclusions of *C. tora* (leaf and Seeds). Henceforth, for better growth performance, nutrient utilization and fish survival, the inclusion level of (25%) of *C. tora* (leaves and seeds) could be used as a supplement in the diet of catfish. However, the mild/little stress placed on the health of the catfish at initial stages of the treatment when fed at high percentage levels of inclusions might be as a result of anti-nutrients influence, more research should be carried out on the uses of *C. tora* in order to understand the anti-nutritional factors influences and better utilization of the plant.

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DESCRITIVES STATISTCS

	RBC ($\times$106L^{-1})	WBC ($\times10^3$)	MCV (μ^3)	MCHC (%)	MCH (pg)	PCV (%)	HB (gdl^{-1})	Total
Leaves 0%	4.32	5	9.2	13.5	13.72	23	3.32	10.29
Leaves 25%	4.44	4.2	9.28	14.3	14.86	23.2	3.44	10.53
Leaves 50%	5.4	4.16	9.36	14.24	14.94	23.4	3.48	10.71
Leaves 75%	5.04	3.12	9.92	14.36	14.68	24.8	3.64	10.79
Leaves 100%	5.12	3.04	9.84	15.94	15.94	24.6	3.92	11.2
Seeds 0%	8.4	3.8	9.68	13.34	13.58	24.2	3.28	10.9
Seeds 25%	5.52	4.8	8.96	14.96	14.96	22.4	3.34	10.71
Seeds 50%	5.5	6.36	9.68	15.32	15.3	24.2	3.7	11.44
Seeds 75%	5.04	4.52	9.84	15.44	15.28	24.6	3.76	11.21
Seeds 100%	5.64	4.52	10	15.68	15.56	25	3.92	11.47
Leaves and Seeds 0%	5.94	4.4	9.68	14.42	14	24.2	3.36	10.86
Leaves and Seeds 25%	4.56	3.8	9.04	15.36	15.38	22.6	3.46	10.6
Leaves and Seeds 50%	4.42	4.4	9.76	15.2	15.2	24.4	3.7	11.01
Leaves and Seeds 75%	5.64	3.64	10	15.04	15.02	25	3.76	11.16
Leaves and Seeds 100%	6.02	3.8	9.38	18.26	18.2	23.4	4.28	11.91
Total	5.4	4.24	9.57	15.02	15.11	23.93	3.62	10.99