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Jibrin H
Department of Biotechnology, Nigerian Institute for Oceanography and Marine research, Lagos, Nigeria

Abubakar MY
Department of Fisheries and aquaculture Usmanu Danfodiyo University, Sokoto, Nigeria

Mamman T
Department of Fisheries and aquaculture Usmanu Danfodiyo University, Sokoto, Nigeria

Abubakar M
Department of Fisheries and aquaculture Usmanu Danfodiyo University, Sokoto, Nigeria

Musa M
Department of Fish Tech., Nigerian Institute for Oceanography and Marine research, Lagos, Nigeria

Ahmad H
Federal college of wildlife Management forestry research Institute of Nigeria

Corresponding Author:
Jibrin H
Department of Fisheries and aquaculture Usmanu Danfodiyo University, Sokoto, Nigeria

Haematological indices of *Clarias gariepinus* (Burchell, 1882) fingerlings fed diet containing graded levels of sickle pod (*Cassia tora*) leaf meal

Jibrin H, Abubakar MY, Mamman T, Abubakar M, Musa M and Ahmad H

Abstract
The effects of *Cassia tora* leaf meal on the hematological indices of *Clarias gariepinus* was investigated for a period of 63 days. *C. tora* leaf meal was incorporated in the diet at 0 (control), 3, 6, 8 and 10% in five different diets. *C. gariepinus* (mean weight 2.17±0.01g) were randomly distributed in to concrete tanks at 20 fish/tank in triplicate treatments. The results of the hematological parameters obtained, revealed significantly low values of white blood cell (WBC), mean corpuscular hemoglobin (MCH), Mean platelet (MPV) and platelet-large cell ratio (P-LCR) in fish fed diet containing inclusion levels of the *Casia tora* leaf meal than the control diet. Fish fed diet containing 3% *Casia tora* leaf meal diet recorded the highest pack cell volume (PCV) and hemoglobin (Hb) and were significantly ($P<0.05$) higher than the other dietary treatments including the control diet. Fishes fed the control diet, 6 and 12% *Casia tora* leaf meal diets had a decrease in Red blood cell (RBC) but were not significantly different ($P<0.05$). The highest mean corpuscular haemoglobin concentration (MCHC) was recorded in fish fed with 3 and12% *Casia tora* leaf meal diet but were significantly ($P<0.05$) different from all the dietary treatments including the control, while the lowest values were recorded in fish fed containing 6 and 9% *Casia tora* leaf meal diets and were significantly different ($P<0.05$). Further investigation on the utilization of *Casia tora* leaf meal in fish feeding and adopting appropriate processing methods for the removal of anti-nutritional factors is desirable.

Keywords: *Cassia tora* leaf meal, *Clarias gariepinus*, haematological parameters

Introduction
In Nigeria, greater proportion of fish supply is from the capture fisheries, which do not meet the demand of the ever-increasing population (Madu *et al.*, 2003) [16]. The need to explore aquaculture for massive fish production is imperative (Lovell, 1992) [14]. One of the most important components of aquaculture is fish feeding (Aydin *et al*., 2011). Feed alone has been estimated to account for 40-70% of the cost of aquaculture operations (Pathmasethy, 1983; Olvera-Nova, 1996) [22, 19]. Thus, the importance of efficiency of utilization of the fish feed cannot be over emphasized. Non-Conventional Feed Resources (NCFRs) are feeds that are not usually common in the market and are not the traditional ingredients for commercial fish feed production (Devendra, 1988; Madu *et al*., 2003) [6, 16]. NCFRs are credited for being non-competitive in terms of human consumption and cheaper to purchase (Devendra, 1988) [6]. They are mostly by-products or waste products from agriculture, farm made feeds and processing industries. They may include all types of feed stuffs from animal (silk worm, maggot, termites, earth worm, snails, tadpoles etc.), plant waste (cotton seed meal, soy bean meal, maize bran, rice bran, palm kernel cake, groundnut cake and brewers waste) from plants (duckweed, cajanus and Chaya) waste from animals such as animal dung, offal, visceral, feather, fish silage, bone and blood (Devendra, 1988; Omitoyin and Faturoti, 2000) [6, 20]. All these can be recycled to improve on fish feed production if economically justified and technologically possible (Devendra, 1988; Omitoyin and Faturoti, 2000) [6, 20].
Sickle pod (*C. tora*) is a common herbaceous annual occurring as a weed, which belongs to the family Leguminaceae. The plant is extensively used in traditional medicine in tropical and warm subs tropical countries (Perry, 1980) [23]. It has been reported that the application of paste of leaves of this plant is effective in the treatment of some chronic diseases and skin ailments (Perry, 1980) [23]. The powdered form of the plant leaf was included in the diet of day old chickens and the results recorded higher growth performance (Ayssiwede *et al*., 2011) [2].

Bloods cells responses are important indicators of changes in the internal or external environment of fish and these changes depend on fish species, age, sexual maturity and diseases (Golovina, 1996; Luscova, 1997) [10, 15]. Blood is a good indicator to determine health condition of an organism (Joshi *et al*., 2002). The leaves contain an appreciable amount of nutrients, such as crude protein (27.40%), crude lipids (3.80%), crude fibre (16.8%), ash (15.20%), Nitrogen free extract (36.80%) and metabolizable energy (ME) of 2573.80kcal/kg (Ayssiwede *et al*., 2011) [2]. The present study aimed at investigating the potentials of using *C. tora* leaf meal in the diet of *Clarias gariepinus.*

Materials and Methods

Experimental Site

The experiment was conducted at the Teaching and Research Fish Farm of the Department of Fisheries and Aquaculture at the main campus of Usmanu Danfodiyo University, Sokoto. The site is located in Sudan Savanna vegetation zone of Nigeria on latitude 13^0 07' 78'' N and longitude of 5^0 12' 25'' E and on 275m above sea level (Google, 2011) [12]. The area is characterized by a long dry season which start from October to May, with cool dry air during the harmattan (November- February), and hot dry air during March – May. Raining season start in June and ends in September. Annual rainfall in the area ranged from 500 to 724 mm (Mamman, 2000) [17]. The mean relative humidity range between 14.9% and 40% in March and June, respectively. Ambient temperature can reach up to 41 ^{0}C during April and May and may fall below 20^0C during December and January.

Processing of *Cassia tora* Leaf

Cassia tora leaf was obtained from Rofia town in Niger State. The leaf was plucked from the stem, sun dried for 3 days, ground using pestle and mortar, and sieved using 1.18mm laboratory sieve to remove the residues. The fine particles were used in combination with other ingredients to produce pelleted feed.

Procuring and Processing of other Feed Ingredients

The other feed ingredients used in the diet were maize, groundnut cake (industrial), blood meal, bone meal. They were sourced within Sokoto metropolis. Fishmeal (Danish), lysine, methionine, and vitamin premix were purchased from Agro- tech., Minna, Niger State. The other feed ingredients that formed the ration included Groundnut Cake, fishmeal, Maize, Blood meal, Bone meal, Methionine, Lysine, Vitamin Premix, Palm Oil and table Salt.

Experimental Diets

Five diets containing varying levels of *C. tora* leaf meal at 0 % (Diet 1), 3% (Diet 2), 6% (Diet 3), 9 % (Diet 4) and 12% (Diets 5) were formulated following Pearson Square Method. The diets were isonitrogeneous (each containing 45% crude protein) and isocaloric (each containing 3,478.60kcal/kg metabolizable energy). The appropriate quantities of ingredients in each diet were weighed and mixed thoroughly using electric feed mixer (Kenwood). Each diet was thereafter mixed with warm water to make dough. The mixed dough was subjected to pelleting using an Electric Feed Pelletizer (50kg capacity/hour) and 2mm disk diameter. The pelleted feeds were sundried and broken into smaller sizes appropriate to the fish and size stored until the commencement of the feeding trial. Table 1 shows the gross compositions of experimental diets.

Table 1: Gross composition of experimental diets

		Experimental	Diets		
Ingredients	**I (0%CLM)**	**II (3% CLM)**	**III (6% CLM)**	**IV (9%CLM)**	**V(12% LM)**
Maize	14.11	12.39	10.66	8.95	7.20
GNC	39.69	39.06	38.42	37.78	37.15
Fish meal	29.77	29.29	28.82	28.33	27.86
CLM	0.00	3.00	6.00	9.00	12.00
Blood meal	9.92	9.76	9.61	9.44	9.29
Bone meal	2.25	2.25	2.25	2.25	2.25
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Palm oil	2.00	2.00	2.00	2.00	2.00
Salt	0.25	0.25	0.25	0.25	0.25
Methionine	0.50	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50	0.50
TOTAL	100.00	100.00	100.00	100.00	100.00
Calc. ME (Kcal/kg)	3,480.06	3,479.47	3,478.99	3,477.61	3,476.89

"GNC" Groundnut Cake, "ME" Metabolizable energy, "CLM" *Cassia tora* leaf meal

Experimental Fish Management

Experimental fish in each concrete tank were fed at 5% body weight for 9 weeks of feeding period. The fish were fed two times daily. The tanks were cleaned, and uneaten feeds together with faecal residues were siphoned out before feeding. Water levels were maintained

Blood collection and Analysis

At the end of the experiment, samples of fish from all the experimental treatments were subjected to hematological analysis to determine the effect of the test ingredient on the fish.1.5 milliliters (ml) of blood was collected from the caudal peduncle as described by (Joshi, 2000) [13]. Hemoglobin was estimated by cyanomethemblobin, white blood cells (WBC) were counted by Neubauer's improved hemocytometre using Hyem's, and Turk's solution as diluting fluid method as described in (Stoskopf, 1993) [26]. Red blood cell (RBC) and packed cell volume (PCV) were estimated as described in (Blaxhall and Daisley, 1973) [3]. Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentrate (MCHC) and mean cell volume (MCV) were calculated using standard formulae as described by (Dacie and Lewis, 1991) [5] and (Joshi, 2000) [13].

Statistical Analysis

The data on nutrient composition of *C. tora* leaf meal,

phytochemical test, growth performance, nutrient utilization, hematological parameters, water parameters and economic indices obtained were subjected to analysis of variance (ANOVA) and the treatment means were separated using Duncan Multiple Range Test (Steel and Torrie, 1980) [25]. The analysis was carried out using SPSS version 20.0.

Results and Discussion

The results of the hematological parameters of fish after the experiment are presented in Table 2. Some values of PCV, RBC and Hb in fish fed diet containing the leaf meal were not within the acceptable range. This indicating that the leaf meal is toxic to *C. gariepinus* and are not safe for fish feed. Haematological components of blood are also valuable in monitoring feed toxicity especially with feed constituents that affect the formation of blood in culture fisheries (Oyawoye and Ogunkunle, 1998) [21].

White blood cells (WBC) and lymphocytes results recorded in this study showed a decreased as the level of *C. tora* leaf meal increased in the diet. The highest value of 91.57±1.00x10^9/l for WBC was recorded in fish fed control diet. The lymphocyte count showed that the highest value of 96.90±0.10% was jointly recorded in fish fed the diets containing 0% and 6% *Cassia tora* leaf meal. White blood cells (WBC) and lymphocytes are the defense cells of the body (Douglas and Jane, 2010) [8], demonstrated that the amount has implication in immune responses and the ability of the animal to fight infection. High WBC count is usually associated with microbial infection or the circulating system (Oyawoye and Ogunkunle, 1998) [21]. The value range of 22.17±0.80 to 91.57±1.00 x10^9/l recorded in this study for WBC was higher compared to 16.13 × 103 to 16.39 × 103 mm-3 reported by (Sotulo and Faturoti, 2009) [27]. The decrease in the WBC and increase in lymphocytes as *Cassia tora* leaf meal increased in the diet could be resulting from feed toxicity and might also environmental factors could affect this, because there is higher values in the control diets due to the infection was observed in the experimental site.

The packed cell volume (PCV) range 10.0±0.05 to 26.40±0.81% observed in this study is not within the range of 20 to 50% reported by (Pietse *et al*, 1981) [24] and rarely do values above 50% being reported (Clarks *et al*, 1976; Etim *et al*., 1999) [9, 4] Though, a decrease was observed in the level of PCV as the level of *C. tora* leaf meal increased in the diet. The decreasing trend observed in the PCV of this study may be attributed to the presence of some anti-metabolites such as tannin and phenol in *C. tora* leaf meal.

The Red blood cell (RBC) was highest in fish fed diet II with 3% CLM inclusion, the value were 2.38x10^{12}/l. This was significantly higher than the other dietary treatments including the control diet but lowered than that obtained in (Aydin 2011). However, there was decreased hematological parameter such RBC in fish fed diet V (12% CLM) than the other dietary treatments. This was an indication that blood was lost in fish fed those diets compared with what was reported by (Dienye, 2014; Mamman *et al*., 2013) [7, 18] when fed *C. gariepinus* with *M. Oleifera* leaf meal and calabash seed cake respectively.

Table 2: Haematological parameters of *C. gariepinus* fed the experimental diets

		Experimental Diets			
Parameters	**1 (0%)**	**II (3%)**	**III (6%)**	**IV (9%)**	**V (12%)**
White blood cell (10^9/ml)	91.57±1.00^a	70.27±1.22^b	17.77±0.80^e	46.68±0.5^c	34.65±0.52^d
Pack cell volume (%)	14.90±0.60^c	26.40±0.8^a	11.70±0.50^d	17.80±0.19^b	10.00±0.05^e
Red blood cell (10^{12}/ml)	1.17±0.20^b	2.38±0.70^a	0.90±0.10^b	1.55±0.10^{ab}	0.85±0.40^b
Hemoglobin (g/dl)	59.00±1.00^a	7.90±0.30^b	3.00±0.10^d	5.80±1.20^c	4.30±0.5^d
Mean corpuscular hemoglobin concentration (%)	39.40±0.40^b	30.00±1.00^a	25.30±0.30^e	32.60±0.40^c	42.90±0.80^a
Mean corpuscular Hemoglobin (pg)	50.40±0.50^a	33.30±0.60^c	33.330±0.60^c	37.60±0.10^b	50.40±0.42^a
Mean corpuscular volume (fl)	127.90±0.90^b	110.90±1.00^e	131.2±0.25^a	115.20±0.60^d	117.60±0.90^c
Red cell distribution width CV (%)	14.90±0.88^b	17.00±1.00^a	16.70±0.20^a	12.00±0.80^c	14.10±0.70^b
Platelet count (10^9/l)	93.00±1.00^c	171.00±1.00^b	76.00±0.10^d	261.00±0.40^a	19.00±0.05^e
Mean platelet volume (fl)	9.90±0.40^a	8.60±0.50^b	9.70±0.80^a	6.90±0.20^c	7.90±0.10^b
Platelet distribution width (%)	10.60±0.50^b	11.40±0.20^a	11.50±0.30^a	11.70±0.60^a	11.50±0.40^a
Plateletcrit (%)	0.09±0.01^{bc}	0.25±0.19^a	0.07±0.01^{bc}	0.18±0.01^{ab}	0.15±0.01^c
Platelet-large cell ratio (%)	31.30±0.62^a	22.60±0.10^c	29.40±0.10^b	11.80±0.90^d	10.20±0.20^e
Red cell distribution width SD (fl)	73.60±0.80^b	68.90±0.60^c	95.20±0.02^a	58.20±0.05^e	66.70±0.45^d
Granulocyte cell percentage (%)	0.40±0.01^b	0.70±0.10^{ab}	1.60±0.80^a	0.90±0.02^{ab}	1.60±0.70^a
Intermediate cells percentage (%)	5.40±0.7^{ab}	5.9±0.20^a	1.50±0.40^d	2.68±0.07^c	4.5±1.00^b
Lymphocyte percentage (%)	94.17±1.2^b	93.45±0.80^b	96.90±0.10^a	96.40±0.10^a	93.90±0.90^b

Means in rows having same letters are not significantly different (P >0.05) Not: pg = picogram fl = femtolite

Conclusion and recommendation

The study concluded that unprocessed *C. tora* leaf meal could not be used in the fish feed and it is recommended that further investigation on the utilization of *C. tora* leaf meal in fish feed with respect to more appropriate processing methods and potent means of removing the anti-nutritional factors to enhance feed palatability and acceptability to produce healthy fish.

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