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Suitability of discarded cashewnut (*Anacardium occidentale*) meal as replacement of soybean meal (*Glycine max*) in the diet of juvenile African catfish *Clarias gariepinus* (Burchell, 1822)

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ABSTRACT

The search for alternative protein sources in aquaculture nutrition is still trending as the use of costly traditional feed stuffs in aquaculture diet formulation is no longer sustainable. The present study investigated the effects of partial and total dietary inclusion of cashewnut meal on growth, haematology, carcass composition, serum biochemistry and intestinal histology of juvenile African catfish *Clarias gariepinus* (Burchell, 1822). Soybean meal (SBM) was substituted with cashewnut meal (CM) at 0, 50 and 100% in formulated fish diets. A total of 135 juveniles of *C. gariepinus* (30.25± 1.02 g; 16.08±0.34 cm) were randomly assigned to three dietary treatments (0, 50 and 100% CM based diets) at a stocking density of 45 fish per treatment and further randomised into triplicates of 15 fish per replicate. The feeding trial lasted for 56 days and fish were fed twice daily at 5% body weight. Statistical analysis was carried out for all the parameters evaluated in this study. Fish group fed 50% CM based diet had the best growth performance and haematological profile when compared to 100% CM fish group and the control group. Insignificant changes in serum biochemical parameters were observed in CM fed fish groups when compared to the control. Histological examination of fish intestinal morphology revealed no adverse changes in the cellular structure of mucosal layer and villi in 50% CM fed fish, while mild histo-morphological changes were observed in 100% CM fed fish group. Significant increases in villi length and weight were observed in fish group fed CM based diets when compared to the control. The findings of the present study revealed that partial replacement (50%) of soybean with cashewnut meal improved growth performance and haematological profile of *C. gariepinus.*

Keywords: *Anacardium occidentale*, Alternative protein sources, Cashewnut meal, *Clarias gariepinus,* Growth performance Haematology, Histology, Intestine

Introduction

The success of aquaculture enterprise is largely dependent on several factors including feed. Feed and feeding have been noted to contribute up to 60% of total fish production costs indicating that feed is obviously a major factor in aquaculture production (Sogbesan and Ugwumba, 2008; Iheanacho *et al.*, 2018). A major constraint in aquaculture production is the expensive nature of most protein based feed ingredients such as fish meal and soybean meal (Ogunji *et al.*, 2008). High demand for soybean by several food industries culminates to its seldom use in aquaculture diet formulation (FAO, 2009). Recently, research on alternative low cost plant and animal protein sources in aquaculture diets has been intensified (Ogunji *et al*., 2008; Iheanacho *et al.,* 2018).

Cashewnut (*Anacardium occidentale)* is an important crop with high export value and also a good plant protein and vitamin source (Akande *et al.,* 2015). Cashew crop is indigenous to Brazil and was introduced to East and West Africa, India and Portuguese in the early 16th century (Frankel, 1991). The crop is now widely cultivated and distributed worldwide (Akinhanmi *et al.,* 2008, Madjitol-Betoloum *et al.,* 2018). On the global scale, cashew is ranked the third most produced edible nut (Salomon *et al.,*2018) and traded for its nutritional value (John *et al.,*2017). World production of cashewnut grew from 125, 000 t in 1955 to 3,971,046 t by 2017 (FAO, 2019). Cashewnut is rich in protein and contains all the essential amino acids (Nambiar *et al.,* 1990). It is available and can be accessed all year round (Akande *et al.,* 2015).

Large quantities of this crop are thrown away during processing due to breakage, bruises and physiological deformities which invariably affect the taste. Akande *et al.* (2015) reported that 30% of the nuts are likely to be lost annually in a similar way at the industrial processing level. This unfortunate situation has been reversed as the discarded nuts are now processed and added to poultry diets. Significant growth rate and feed utilisation efficiency have been reported in chickens fed cashewnut incorporated diet (Akande *et al.,* 2015). The best growth performance in terms of weight gain, final weight and protein efficiency ratio were observed in chickens and pigs fed cashewnut meal based diet when compared to the control group (Piva *et al.,* 1971; Fetuga *et al.,* 1974). Akande *et al.* (2015) reported significant increase in weight gain, feed intake and final weight of chicken fed discarded cashewnut based diet with reference to the control. To the best of our knowledge, no study regarding the use of cashewnut meal as alternative plant protein source in aquaculture has been carried out so far. The present study evaluated the suitability of discarded cashewnut meal as repalcement of soybean meal in diet of juvenile African catfish *Clarias gariepinus* (Burchell, 1822) in order to ascertain its effect on growth, haematology, biochemical parameters and intestinal histo-morphology.

Materials and methods

Experimental fish and rearing conditions

Apparently healthy African catfish juveniles *Clarias gariepinus* (N = 135, average weight 30.25±1.02 g) were used for this experiment. The fish specimens were obtained from the fish farm of the Department of Fisheries and Aquaculture, Alex Ekwueme Federal University Ndufu Alike Ikwo, Ebonyi State, Nigeria. Fish specimens were transferred to the wet laboratory of the Department of Fisheries and Aquaculture. Fishes were acclimated in 9 glass aquaria (15 nos. per tank); each filled with 50 l dechlorinated tap water for 14 days. Fish were fed commercial diet (Aqualis, USA 48% crude protein) at 5% of the fish biomass. As the experiment was conducted under semi-static system, water in the experimental tanks was always replaced with fresh dechlorinated tap water every three days (Bello *et al.,* 2014) to avoid fouling. Experimental tank water was maintained at temperature : 26.21±0.21°C, pH: 6.85-7.34, dissolved oxygen: 6.42± 0.14 mg l^{-1}, ammonia: 0.12 ± 0.02 mg l^{-1} and nitrite: 0.15 ± 0.05 mg l^{-1}, with a controlled photoperiod (12 h light: 12 h darkness) in the laboratory.

Preparation and processing of Anacardium occidentale (cashewnut) meal

Discarded cashewnuts (broken) were collected from Embik Cashew Nut Industry located at Umuorii Uratta, Owerri, Imo State, Nigeria. The nuts were sieved to remove any form of contamination. The cashew nuts were air dried at 29°C for 48 h. The nuts were powdered using a mechanical grinder (LM2421EG, Moulinex, France), packaged into an air tight container and stored in the refrigerator at 4°C until used.

Experimental diet

Other feed ingredients used for diet formulation were purchased from a nearby local market in Abakaliki, Nigeria. Feed ingredients used include fishmeal, SBM wheat bran, maize, vitamin/mineral premix, palm oil, sodium chloride (salt), starch and bone meal. Soybean was toasted at 120°C for 70 min using a hot air oven (Memmert W., Germany) in order to reduce anti-nutritional factor (ANF) level (Ogunji *et al.,* 2008). Percentage inclusion levels of ingredients was calculated using the conventional Pearson's square method (De Silva and Anderson, 1995). Diets for the experiment were formulated having CM at inclusion levels of, 0%, 50% and 100% to replace soybean meal (Table 1). The ingredients of the experimental diet were powdered using a fabricated milling machine and were mechanically mixed, then pelletised and oven dried at 65°C for 5 h, then stored at 4°C in the refrigerator until use.

Proximate analysis

Proximate analysis of SBM, Cashewwnut meal (CM), experimental diets and fish (carcass) before and after the experiment were carried out to ascertain their nutrient status (Tables 1; 3). A total of 10 g of the formulated diets were sent to Nutrition laboratory of the International Institute of Tropical Agriculture (IITA), Ibadan Oyo State, for proximate analysis. Analysis was done following the procedure described by AOAC (2000).

Experimental design

The experiment lasted for 56 days, during which fish were randomly assigned to three dietary treatments containing 0 (control), 50 and 100% CM. Each treatment contained 45 fish and was further randomised into triplicate experiment of 15 per replicate in complete randomised design (CRD). Fish were fed two times daily (08 00 and 16 00 hrs) at 5% of total body weight. Uneaten feed pellets were removed with hand scoop net. Fish were weighed fortnightly to estimate their weight and adjustment was made regarding the amount of feed administered to the fish.

Growth performance

Initial weight of fish was estimated using electronic (S. Mettler, China). After 56 days of feeding trial, the final balance weight was recorded which form the basis for

Table 1. Proximate composition (% dry matter) of cashewnut meal (CM) and soybean meal (SBM) and experimental diets

Ingredients	0% CM		50% CM		100% CM
Fishmeal (Salmon fish)	300.00		300.00		300.00
Soybean meal	250.00		125.00		0.00
Wheat bran	150.00		150.00		150.00
Maize	150.00		150.00		150.00
Cashew nut	0.00		125.00		250.00
Premix*	40.00		40.00		40.00
Bone meal	30.00		30.00		30.00
Palm oil	40.00		40.00		40.00
Iodized Salt	20.00		20.00		20.00
Binder(cassava starch)	20.00		20.00		20.00
Total	1000.00		1000.00		1000.00
Parameter	Cashew nut	Soybean (toasted)	0.00% CM	50.00 % CM	100% CM
Crude protein (%)	31.15	40.14	41.05	40.89	40.15
Crude fibre (%)	3.09	3.23	4.76	3.85	4.05
Crude fat (%)	30.98	12.55	14.31	17.94	19.54
Ash (%)	4.97	10.91	9.14	7.25	7.13
Moisture (%)	10.42	7.85	8.18	9.03	8.86
Dry matter (%)	89.58	92.15	91.82	90.97	91.14
NFE** (%)	19.39	25.32	22.56	21.04	20.27

Composition of vitamin and mineral mixture (per kg) : Vitamins: A - 10,000 IU; D - 3,3,000 IU; E - 20,000 mg; K - 1000 mg; B6 - 1500mg; B12 - 10.0 mg; Niacin - 10,000 mg; Pantothenic acid - 1000 mg; Riboflavin - 80 mg; Thiamin - 30 mg; Folic acid - 1000 mg; Minerals: Fe - 60 mg; Mn - 80 mg; Mg - 100 mg; Cu - 80 mg; Zn - 50 mg; Se - 100 mg; Choline - 50 mg. **NFE = Nitrogen Free Extract, Dry matter = 100 - (Crude protein + Crude fat + Crude fibre + Ash + NFE).

measuring the following growth indices as described by Windell (1978) and Iheanacho *et al.* (2018):

$$\text{Weight gain (\%) (WG\%)} = \frac{Wt_2 - Wt_1}{N}$$

where, Wt_1 = Initial mean weight of fish at time t1; Wt_2 = Final mean weight of fish at time t2; N = No. of days

$$\text{Protein intake} = \frac{\text{\% Protein in feed x Total diet consumed}}{100}$$

$$\text{Specific growth rate (SGR)} = \frac{100\,(\log_e Wf - \log_e Wi)}{\text{Time (days)}}$$

where, Wf = Final average weight at the end of the experiment; Wi = Initial average weight at the beginning of the experiment

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Amount of feed consumed}}{\text{Weight gain of fish}}$$

Haematology and serum biochemistry

Three fish per replicate were sampled at random for blood collection for haematological and serum chemistry analysis. Fish were caught with scoop net and anaesthessed with MS 222 (50.0 mg l^{-1}) to minimise stress. Blood samples were obtained following Bello *et al.* (2014). Under gentle aspiration, 3 ml of blood were collected from caudal vein and transferred to sterile tube for serum collection, while heparinised syringe was used for collecting blood for haematological analysis. Red blood cells (RBC) and white blood cells (WBC) were counted manually using Neubauer haemocytometer after diluting blood samples by adding Hayem solution for RBC and Turk solution for WBC (Dacie and Lewis, 2011). Packed cell volume (PCV) was measured using standard heparinised microhaematocrit capillary tubes (75 mm) by centrifugation at 7000 g for 15 min. Haemoglobin content (%) was measured spectrophotometrically at 540 nm by cynomethaemoglobin method (Dacie and Lewis, 2011). Red cell indices (mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) were calculated as described by Dacie and Lewis (2011).

Serum biochemistry

The clotted blood in the plain tubes were transferred into clean dry centrifuge tubes and centrifuged at 3000 rpm for 15 min. The supernatant (serum) formed after centrifugation was collected using Pasteur pipette and transferred into a plain plastic tube for biochemical analysis. Biochemical parameters such as total protein, glucose, total triglyceride, cholesterol content, serum enzymes *viz*., alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST)), blood urea nitrogen (BUN) and creatinine levels were determined using a semi-automated biochemistry analyser (Erba Chem 7, India).

Histological analysis

Histological examination of fish intestine was carried out following the procedure described by Belelander and Ramaley (1979), at the histology laboratory of the Department of Anatomy, Alex Ekwueme Federal University, Ndufu Alike, Ebonyi State, Nigeria. At the end of the experiment, two fish per treatment were collected at random with hand scoop net and sacrificed for tissue collection. Fish intestine was excised and rinsed in physiological saline to remove traces of blood and fixed in Bouin's fluid and processed as per Belelander and Ramaley (1979), before embedding in paraffin wax of melting point 56°C. Sections (5 µm) were cut using microtome and stained with haemoxylin and eosin (Belelander and Ramaley, 1979). After staining, villi length and villi width were photographed and measured using an image processing and analysis system (ZEN 2012 SP2) (Ozel *et al.,* 2018).

Statistical analysis

Data obtained from the examined parameters were subjected to one-way analysis of variance (ANOVA), using SPSS IBM, version 20.0. Significant difference was declared at 5%, using Tukey post-hoc test. Also part of the results were presented in bar charts using Excel, version 16.0.

Results and discussion

Results on nutrient composition of the experimental diets revealed that crude protein ranged from 40.89-41.05%; ash 7.13-9.14%; moisture 8.18-9.03%; fat 14.31-19.54% and NFE 20.27-22.56%. In the present study, cashewnut has been revealed to contain up to 31.15% crude protein, 4.97% ash, 3.09% crude fibre, 30.98% fat and 19.39% NFE (Table 1). Akande *et al.* (2015) reported that full fat cashewnut contains 40.23% crude fat, 22.10% crude protein and ash content of 3.73%. As per Segun *et al.* (2009) cashewnut contains 27% crude protein, 34.95% fat, 8.59% moisture, 4.41% ash, and 1.90% fibre. Ogungbenle and Afolayan (2015) also reported 26.1% protein, 2.91% ash, 42.90% fat, 5.90% moisture and 3.11% fibre in cashewnut.

Data on growth performance of *C. gariepinus* fed CM supplemented diets are presented in Table 2. Fish fed 50% CM based diet had significantly higher values in terms of final weight, weight gain and specific growth rate**,** followed by the control and 100% CM fed fish group. Data on fish survival revealed that there was no significant difference between the control and fish group fed CM based diets. This could be attributed to the nutrient content of cashewnut, diet palatability and acceptability by fish. Cashewnuts have been reported to be highly nutritious and contain all the essential amino acids, vitamins and mineral elements (Sogunle *et al.,* 2009; Madjitol-Betoloum *et al.,* 2018). Akande *et al.* (2015) reported significant increases in final weight and weight gain in chicken fed with full fat cashewnut meal based diet with reference to the control.

Ojewola *et al.* (2004) reported significant increase in final weight, weight gain and daily weight gain of broiler fed cashewnut meal based diet especially at 50% dietary inclusion level with reference to the control. Examination of the villi morphometry revealed that CM fed groups had significantly higher values than the control (Table 2). The gastrointestinal morphology is related to feeding habits such as food components, food intake frequency along with body size, shape and species (Khojasteh *et al.,* 2009). The intestines are among the most important organs of fish charged with digestion and absorption of ingested food. Intestinal villi is responsible for the absorption of nutrients and pumping of absorbed nutrients into the blood or lymph (Ozel *et al.,* 2018). Villi length (VL), width (VW) and length to width ratio (VL/VW) were significantly influenced by CM diets when compared to the control. Increase in villi

Table 2. Growth performance and distal villi morphology of *C. gariepinus* juvenile fed different dietary percentage levels of CM based diets

Parameters	Control (0.0%) CM	50% CM	100% CM
Initial weight (g)	29.77±0.34^{a}	30.04±0.03^{a}	30.01±0.00^{a}
Final weight (g)	87.75±0.36^{b}	89.60±0.70^{a}	81.68±2.33^{c}
Weight gain (g)	57.98±0.27^{b}	59.55±0.68^{a}	51.67±2.34^{b}
SGR (g day^{-1})	1.57±0.02^{b}	1.95±0.01^{a}	1.37±0.07^{b}
Protein intake (g)	0.77±0.10^{a}	1.29±0.26^{a}	1.17±0.09^{a}
FCR	2.59±0.01^{a}	1.43±0.50^{b}	2.02±0.10^{a}
Survival	100.00±0.00^{a}	100.00±0.00^{a}	90.00±0.01^{a}
Villi morphology			
VL (µm)	545.24±94.05^{b}	689.12±97.32^{a}	673.31±101.54^{a}
VW (µm)	153.38±47.05^{b}	167.37±36.23^{a}	165.21±45.04^{a}
VL/VW	3.64±1.06^{b}	4.27±1.24^{a}	4.18±1.63^{a}

Means (n=3) ± (SE) within rows with different superscripts are significantly different ($p< 0.05$).

size may suggest proper absorption of diets (Ozel *et al.,* 2018). Zaki *et al.* (2015) reported increase in villi length of seabass (*Dicentrarchus labrax*) fed chitosan incorporated diet. Ozel *et al.* (2018) reported significant increase in villi length and width of Black Sea trout (*Salmo labrax*) fed increased level of dietary protein. Results on carcass compositions of fish fed CM based diets are presented in Table 3. Significant decrease in crude protein was observed in fish group fed 100% CM, but had the highest crude fat content than the 50% CM fish group and control. However, no significant difference was observed between the control and CM fed fish groups in terms of crude fibre, ash and NFE contents. Moisture content was significantly higher ($p<0.05$) in the initial fish, followed by the control group, when compared to CM fed fish groups while dry matter content was observed to be significantly reduced in the control group when compared to the CM fed fish groups. The findings of the current study suggest that cashewnut meal added substantial amount of nutrient to fish flesh especially when compared to the initial carcass value for protein. This study also revealed that fish fed CM based diets had significantly high fat content than the initial and control fish, which could be due to the fact that cashewnut is also rich in fat and oil (Segun *et al.,* 2009).

Haematological parameters are important biomarkers for evaluating the health conditions of animals as regards to pathological situations and physiological functions (Iheanacho *et al.,* 2018; Yaji *et al.,* 2018). Haematological data of *C. gariepinus* fed CM based diets are presented in Table 4. Significant increase ($p<0.05$) in PCV, Hb, and RBC contents were observed in fish fed 50% CM when compared to 100% CM fish group and the control, thus suggests that CM based diets fed to fish did not compromise haematopoiesis, erythrocytogenesis and biosynthesis of haemoglobin molecules (Yaji *et al.,* 2018). Insignificant changes were observed in WBC counts of fish groups fed CM based diets when compared to the control group, suggesting that CM based diets fed to fish did not elicit stress on fish. Red cell indices (MCV, MCH and MCHC) are important bioindicators of anaemic conditions in animals (Dacie and Lewis, 2011; Iheanacho *et al.,* 2018). Alterations (increase and decrease) in red cell indices often indicate macrocytic and microcytic anaemia (Dacie and Lewis, 2011). The findings of this study revealed that there were no significant changes in red cell indices of CM fed fish, thus implies that CM based diets fed to the fish did not induce any of the above mentioned anaemic conditions. Insignificant changes were observed in leukocyte differential counts (neutrophils, lymphocytes, basophils, monocytes, eosinophils) of CM treated groups when compared to the control. This also suggests that CM did not elicit any form of stress in fish.

Biochemical parameters are important bioindicators for evaluating metabolic and physiological functions in animals (Yaji *et al.,* 2018). Serum biochemical profile of *C. gariepinus* fed CM supplemented diets are presented in Fig. 1. Insignificant changes ($p> 0.05$) were observed in all the parameters evaluated with reference to the control. Akande *et al.* (2015) reported insignificant changes in protein, cholesterol, albumin and globulin values of chicken fed cashewnut meal when compared to the control group. Shao-Wei-Zhai *et al.* (2014) reported significant decreases in total protein, glucose, cholesterol and triglyceride levels in tilapia (*Oreochromis niloticus*) fingerling fed grape seed meal based diets when compared to the control. Serum enzyme activity (ALP, AST and ALT) and the levels of energy metabolites (triglycerides and cholesterol) of fish are considered as essential diagnostic tools (Coz-Rakovac *et al.,* 2005), used in estimating the health status of fish (Christofilogiannis, 1993). Present study revealed that there were no significant changes in serum enzyme levels of fish groups fed CM based diets when compared to the control.

Intestine of fish comprises mucosa, submucosa, muscularis and serosa layers (Mokhtar *et al.,* 2015). According to Ozel *et al.* (2018), mucosal layer is composed of epithelium, lamina propria and muscularis mucosa (Mumford *et al.,* 2007) and could be influenced by biotic and abiotic factors (Khojasteh, 2012) such as species and diet (Raskovic *et al.,* 2011; Iqbal *et al.,* 2018), feeding pattern, body shape and age (Cao *et al.,* 2011). Intestinal

Table 3. Carcass composition (% dry matter) of *C. gariepinus* fed different dietary percentage inclusion levels of CM based diets

Parameters	Initial status	Control (0.0%)	50% CM	100% CM
Crude protein	19.02±0.01	21.04±0.01^{a}	20.62±0.91^{a}	18.06±0.25^{b}
Crude fibre	3.17± 0.01	3.29±0.03^{a}	3.85±0.01^{a}	4.30±0.06^{a}
Crude fat	6.04±0.01	9.08±0.01^{b}	15.17±2.74^{b}	19.17±4.32^{a}
Dry matter	39.89± 0.01	43.84±0.03^{b}	49.88±0.01^{a}	52.77±0.14^{a}
Moisture	60.11± 0.01	56.10±0.08^{a}	50.12±0.07^{ab}	47.23±0.01^{b}
Ash	5.02 ± 0.01	6.04±0.01^{a}	5.93±0.75^{a}	5.56±0.25^{a}
NFE*	6.64±1.32	4.45±1.54^{a}	4.31±1.33^{a}	5.68±1.24^{a}

*NFE = Nitrogen Free Extract, Drymatter = 100 - (Crude protein + Crude fat + Crude fibre + Ash + NFE). Means (n=3) ± standard error (SE) within rows with different superscripts are significantly different ($p < 0.05$).

histology of *C. gariepinus* fed CM based diets are presented in Fig. 2. Normal histomorphologic structures of the intestine characterised with well separated villi and normal epithelial mucosa was observed in the control and 50% CM fed fish**,** thus suggests that partial replacement (50%) of soy bean with CM had no adverse implication on the intestinal health of fish, indicating effective absorption and digestion. Evidence of this claim is reflected in the normal cellular structure of the mucosal layer which is responsible for the secretion of digestive enzymes and the peptide hormone known as cholecystokinin (CCK) (Kamisaka *et al.,* 2003; Micale *et al.,* 2014; Iheanacho *et al.,* 2018). CCK hormones play an essential and functional role in the digestive formation in fish and are produced by endocrine cells found in the intestinal mucosa (Kamisaka *et al.,* 2003; Murkshita *et al.,* 2009; Webb *et al.,* 2010). However, mild changes such as fusion of the villi, widening of the lamina propria and erosion of the epithelial mucosa were observed in 100% CM based diet fed fish. The mild histomorphologic changes observed in 100% CM fish could be attributed to the high inclusion level of cashewnut meal in fish diet.

Scarce and costly nature of some conventional protein based ingredients has necessitated the search for alternative animal cum plant protein sources. Based on the results of growth indices observed in the present study, fish fed 50% CM based diet performed best in terms of weight gain, final weight, and specific growth rate and feed conversion ratio. Also 50% CM fish group had best haematological profile (RBC, Hb, PCV) than 100% CM based diet and the control. The findings of the current study revealed that cashewnut meal can serve as an alternative protein source and partially replace soybean meal in the diet of *C. gariepinus.* Study on the cost effectiveness of replacing soybean meal with cashewnut meal is advised as its abundance, accessibility and availability vary from country to country.

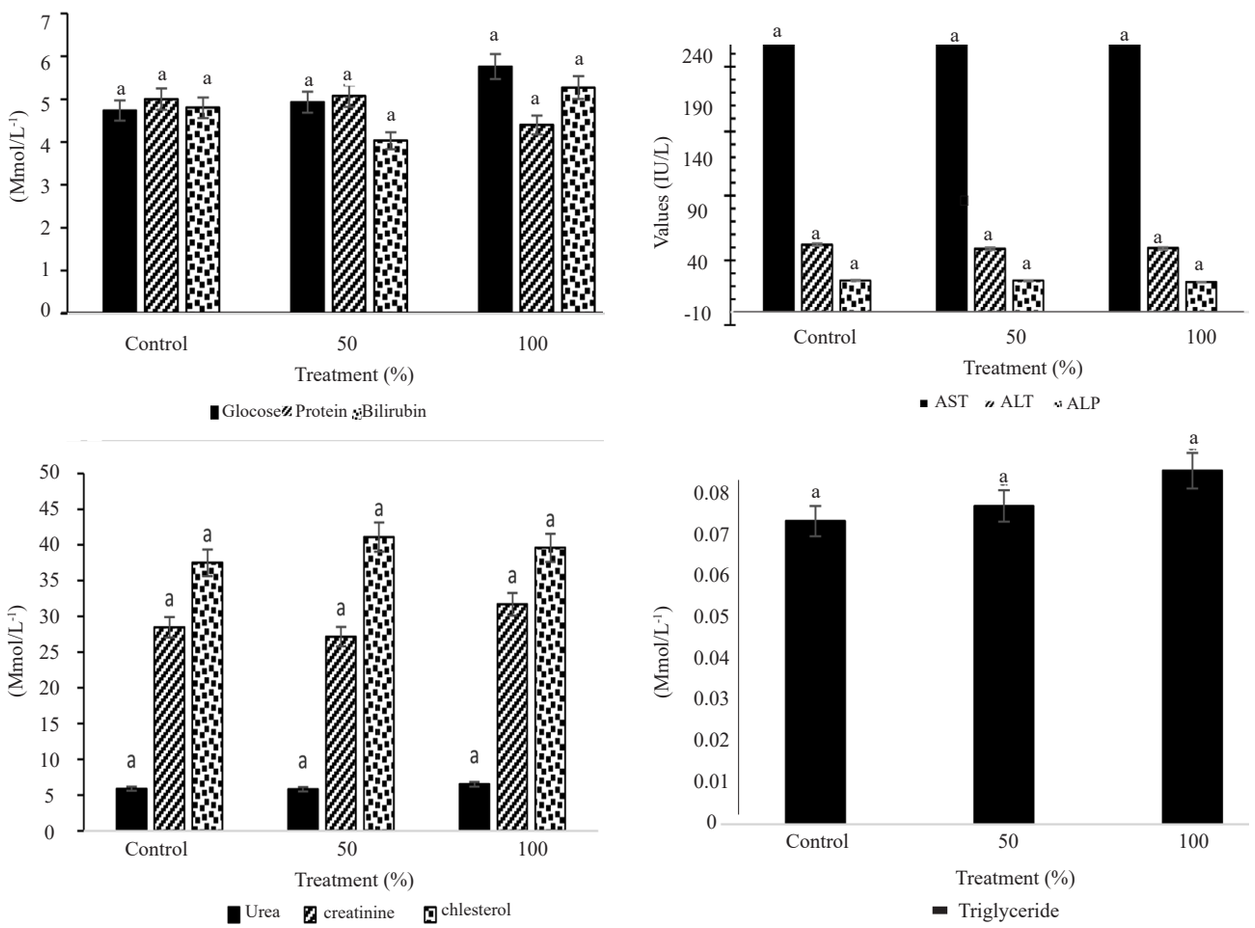
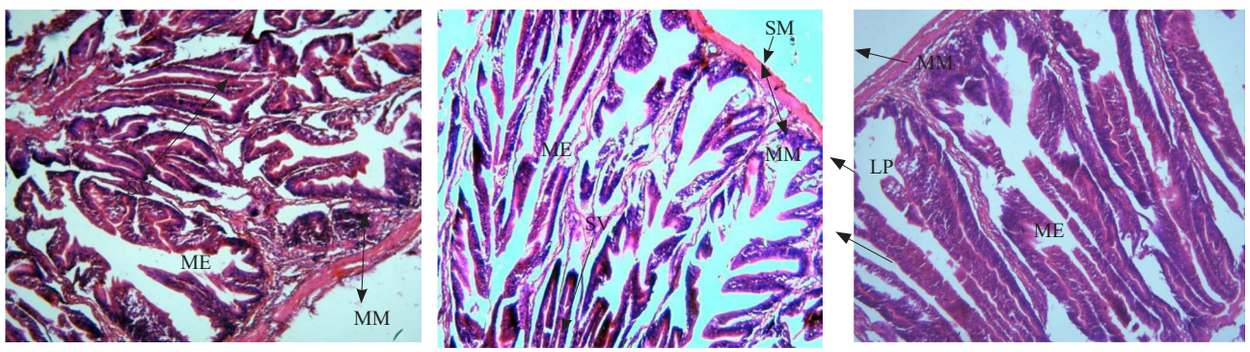


Fig.1. Serum biochemical profile of *C. gariepinus* fed CM based diets for 56 days. Vertical bars with the same alphabet letter denote insignificant difference ($p<0.05$) based on oneway ANOVA.

Table 4. Haematological profile of *C. gariepinus* fed different dietary percentage inclusion levels of CM based diets.

Parameters	Control (0.0 %)	50 % CM	100 % CM
PCV (%)	34.72±0.31^{b}	38.08±0.06^{a}	35.63±0.82^{b}
Hb (g dl^{-1})	11.80±0.50^{b}	14.60±0.76^{a}	11.90±1.02^{b}
RBC (×10 ^{12L})	4.13±0.08^{b}	6.13±0.26^{a}	4.80±0.71^{b}
WBC (×10^{9L})	20.10±1.59^{a}	21.10±1.00^{a}	21.17±2.14^{a}
PLT (%)	124.33±9.28^{a}	116.00±5.57^{a}	118.33±6.36^{a}
MCV (fL)	83.87±0.61^{a}	71.10±3.04^{a}	76.83±6.24^{a}
MCH (pg)	28.53±0.72^{a}	25.93±0.99^{a}	27.37±1.72^{a}
MCHC (%)	34.00±0.82^{a}	36.50±0.89^{a}	35.93±0.71^{a}
Neutrophil (%)	31.00±1.15^{a}	33.33±1.45^{a}	31.33±2.96^{a}
Lymphocytes (%)	65.00±1.53^{a}	63.00±2.00^{a}	64.00±4.16^{a}
Monocytes (%)	3.00±1.00^{a}	3.00±1.15^{a}	64.00±4.16^{a}
Eosinophils (%)	1.00±0.57^{a}	0.33±0.33^{a}	2.00±1.15^{a}
Basophil (%)	0.00±0.00^{a}	0.67±0.33^{a}	0.33±0.33^{a}

Means (n=3) ± standard error (SE) within rows with different superscripts are significantly different ($p< 0.05$) while means with similar superscripts denote insignificant difference ($p > 0.05$). PCV - Pack cell volume, Hb - Haemoglobin, RBC - Red blood cell counr, WBC - White blood cell count, PLT - Platelets count, MCV - Mean corpuscular volume, MCH - Mean corpuscular haemoglobin, MCHC - Mean corpuscular haemoglobin concentration



(a) (b) (c)

Fig. 2. Photomicrographs (H&E, x100) of distal intestinal morphology of *C. gariepinus* fed DCNM based diet and control diet. (a) Control fish, (b) 50% CM based diet fed fish, (c)100% CM based diet fed fish. ME -Mucosa epithelium, SV - Separated villi, MM - Muscuralis mucosa, LP - Lamina Propria

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