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Original Article

Aquaculture by-product meal as a fishmeal replacer in African catfish (*Clarias gariepinus*) diet: Effects on serum biochemistry, innate immune response, and oxidative stress markers

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Abstract: The effect of feeding aquaculture by-product meal (ABP) to African catfish (*Clarias gariepinus*) was investigated in a 56-day feeding trial using serum biochemistry, innate immune response, and oxidative stress markers as indices of assessment. Fishmeal protein in control diets was replaced at a rate of 15, 30, 45, and 60% by aquaculture by-product meal protein. Each experimental diet was randomly distributed into triplicate tanks containing catfish fingerlings (n = 15 fingerlings/replicate, 5.58±0.05 g). The primary haematological parameters (haemoglobin, packed cell volume, red blood cell count) and secondary haematological parameters (MCH, MCV, and MCHC) were similar to the control. The white blood cell count and its differential of the fish group fed ABP meal was numerically higher than control but not significant, except in fish fed D30T that had high lymphocyte count. The platelet count in all the dietary groups was similar. There was no significant variation in some of the serum biochemistry parameters: total protein, albumin, globulin and albumin/globulin ratio, urea, HDL-C and LDL-C. Creatinine values of the D60T-fed group were significantly higher than all other dietary treatment groups including the control. Triglyceride level was statistically similar with control up to 30% replacement level, while there were no significant variations in the cholesterol levels of the blood of *C. gariepinus* fed the different dietary treatments. Except for catalase, there were no significant differences in other oxidative stress biomarkers under study, primarily SOD, GSH, and GPx. Catalase enzyme activities of the fish group fed D30T were statistically higher than other fed groups. Some serum electrolytes, such as calcium and chloride ions of the differently fed fish groups, were not significantly different. Lastly, serum potassium ions were significantly higher among D60T-fed group though statistically similar to D45T-fed group. No stress conditions were recorded among the dietary groups. These results showed that the health status and immunity of African catfish were not degraded by feeding aquaculture by-product meal to the fish.

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Introduction

The global human population increase will reach 9.7 billion by 2050 (United Nations, 2019; United Nations, 2015). This will lead to increased food demand and the need to increase food supply by up to 70% of the current supply capacity (Hunter et al., 2017). Increased animal production to meet man's growing protein demand has drawbacks, including, but not limited to, water scarcity, habitat destruction, and biodiversity loss (Rivera-Ferre et al., 2016; Benton et al., 2018; Michalk et al., 2019). Aquaculture production, being the fastest growing sector in food production, portends the ability to meet a larger percentage of animal-derived protein (FAO, 2018). Thus, the expansion of aquaculture production in recent years is central to sustained human nutrition (Hua et al., 2019). However, the long-term expansion of aquaculture means a reduced reliance on forage fish, which is a key component of fishmeal. Although the proportions of fish meal and fish oil from forage fish in aquafeeds have declined over the last 20 years, they remain key feed components for many carnivorous fish and crustaceans (Turchini et al., 2019). Increased demand for fishmeal and fish oil as raw materials for aquafeed manufacture has been identified as a likely contributor to overfishing and a

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global fisheries disaster (Türkmen, 2019).

Many researchers have reported the viability of replacing fish meal with a variety of plant protein sources (see Tacon et al., 2009), but antimetabolites, and limited expansion potential due to increased pressure on land and water resources, serve as a barrier to the long-term use of plant protein sources as fish feed ingredients (Döös, 2002; Malcorps et al., 2019). Hua et al. (2019) projected that in some years (2025), extra 37.4 million tons of aquafeed would be needed to meet aquaculture production feed requirements. Hence, researching alternative feed ingredients to fishmeal beyond plant protein sources becomes imperative.

Aquaculture by-product meal remains a veritable substitute for conventional fish meal, and more imprtly, it is an animal protein source that is economically viable and sustainable (Gümüş and Aydin, 2013; Kim et al., 2018). The inedible portion of fish, mainly fish viscera, discarded as waste from the fish processing industry could be used as a replacer of fishmeal (Irm et al., 2020). They are generally referred to as aquaculture by-products, i.e., remnants obtained after fish processing for human consumption (Olsen et al., 2014; Stevens et al., 2018). Nearly 10% of globally-produced fishmeal is from aquaculture by-products (Ytrestøyl et al., 2015; FAO, 2018). Fagbenro et al. (2005) reported that 25-35% of waste products could be generated from catfish processing. These can be reduced to aquaculture by-product meal (ABM) after cooking and drying the waste products. The chemical composition of fish visceral differs with processing, species, nutrient intake, sex, age, seasonality, etc. (Huss, 1995). ABM has inferior nutritional contents consisting mainly of reduced crude protein (52-67%), higher lipid (12-23%), and ash content (7-14%) (Goddard et al., 2008; Kim et al., 2014) compared to a fish meal made from wild catches (National Research Council, 2011; Ween et al., 2017). This could restrict its complete replacement for conventional fishmeal. Hua et al. (2019) indicated that a vast potential exists to increase the volume of meals generated from aquaculture by-products due to aquaculture production projected to reach 109 million tons in 2030.

In Nigeria, catfish is the most cultured fish species. Farmers supplement their fish feeding in earthen ponds using fresh fish viscera from fish processing plants without aeration. This could lead to oxygen depletion and pathogenic organisms could also develop due to the decay of uneaten fish viscera. ABM obtained from aquaculture has been successfully used in practical diets of many fish (Goddard et al., 2008; Tacon et al., 2009; Kim et al., 2014; Kim et al., 2018; Irm et al., 2020). Hernández et al. (2004) included fish viscera meal in the diet of shrimp (*Litopenaeus vannamei*), while trout processing waste was also used in gilthead bream (*Sparus aurata*) practical diets (Kotzamanis et al., 2001). Other scientists that have included fish visceral in the practical diets of Asian catfish (*Clarias batrachus*) are Giri et al. (2000) and Gupta and Gupta (2013). As far as we know, a lack of information exists on the use of fish visceral meal in the diet of African catfish (*Clarias gariepinus* Burchell) (Osho et al., 2019; Jimoh et al., 2021). The effect of ABM on serum biochemistry, innate immune response and oxidative stress markers, and the response of African catfish needs to be investigated. Therefore, this study examines the effect of fishmeal substitution, ABM, on serum biochemistry, innate immune response, and oxidative stress markers of African Catfish.

Materials and Methods

Preparation of aquaculture by-product meal and diet preparation: Fresh offal of fish, consisting mainly viscera, was obtained from fish processing facilities of the University of Ibadan fish farm (7°26'29.1"N, 3ᵛ53'59.1"E), and cooked for 25 minutes following procedures to reduce the fat content and eliminate possible pathogen (Saleh et al., 2020). The cooked offal was blended and sieved to produce an ABM after oven-drying it at 50°C for 72 hours. The resulting meal was analyzed for its proximate composition and kept frozen (-4°C) until used for diet formulation (Table 1). Proximate composition of the feed ingredients and feed preparation process were given in our previous study (Jimoh et al., 2021).

Table 1. Ingredients (g kg^{-1} as fed basis) and nutrient composition (g kg^{-1}) of the experimental diets containing ABM (Jimoh et al., 2021).

	Experimental Diet				
Ingredient Composition (g kg^{-1})	Control	D15T	D30T	D45T	D60T
Fishmeal @ 68% crude protein	287.2	244.1	200.9	158.0	114.9
ABM @ 47.83% crude protein	0.00	61.2	122.5	183.7	245.0
SBM @ 38% crude protein	450.0	450.0	450.0	450.0	450.0
Maize @ 10% crude protein	100.0	100.0	100.0	100.0	100.0
Fish Premix*	40.0	40.0	40.0	40.0	40.0
Fish oil	5.0	5.0	5.0	5.0	5.0
Soybean Oil	5.0	5.0	5.0	5.0	5.0
Starch	112.8	946	76.6	58.3	40.1
Proximate Composition (g kg^{-1})					
Moisture	86.7	82.1	77.5	72.9	67.9
Crude protein	391.8	389.6	387.2	385.1	383.9
Crude lipid	143.1	142.7	142.4	142.0	141.2
Ash	71.8	69.4	67.1	64.8	62.4
Crude fiber	20.2	21.5	22.8	24.1	25.5
NFE	286.5	294.7	303.0	311.1	319.2
Energy (kcal/g)	48.2	48.4	48.7	48.9	49.2
Calculated Amino Acid Composition (g kg^{-1})					
Arginine	30.3	28.8	27.3	25.9	24.3
Histidine	9.7	9.6	9.5	9.4	9.3
Isoleucine	18.6	17.7	16.8	15.8	14.8
Leucine	30.7	29.3	27.9	26.5	25.0
Lysine	28.7	27.4	26.0	24.6	23.2
Methionine	8.9	8.4	7.9	7.4	6.9
Methionine + Cysteine	14.1	13.6	13.0	12.5	11.9
Phenylalanine	17.8	17.1	16.4	15.6	14.9
Phenylalanine + Tyrosine	30.9	29.5	28.1	26.7	25.2
Threonine	18.2	17.2	16.2	15.2	14.1
Tryptophan	4.7	4.4	4.2	3.9	3.6
Valine	20.2	19.3	18.4	17.5	16.6

*1 kg Aero-mix® fish premix contains Vitamin A 25,000,000 IU, Vitamin D3 2,000,000 IU, Vitamin E 200,000 IU, Vitamin K 8000 mg, Vitamin B2 20,000 mg, Vitamin C 500,000 mg, Niacin 150,000 mg, Pantothenic Acid 50,000 mg, Vitamin B6 12,000 mg, Vitamin B12 10 mg, Folic Acid 4000 mg, Biotin 800 mg, Choline Chloride 600,000 mg, Cobalt 2,000 mg, copper 4,000 mg, Iodine 5,000 mg, iron 40,000 mg, Manganese 50,000 mg, Selenium 200 mg, Zinc 40,000 mg, Antioxidant 100,000 mg, Lysine 100,000 mg, Methionine 100,000 mg manufactured by Aerobic Integrated Concept limited Km 130, Lagos Ibadan Expressway, Hossanah Bus Stop, opposite Islim Filling Station, P. O. Box 22109 UI post Office , Oyo State, Nigeria

Experimental fish and system: The healthy and actively swimming fingerlings of African catfish (5.58±0.05 g) were procured from a hatchery at Yesha-Yahu Nigeria Ltd., Egbejila, Ilorin, Kwara State, Nigeria and acclimated for 14 days in an intermediate bulk container (IBC tanks) in the laboratory of the University of Ilorin, Nigeria. The fingerlings were fed a commercial pelleted diet (1.8 mm Skretting Feed Production Co. Inc) for African catfish. After acclimation, 225 fingerlings were randomly stocked into fifteen 60-L rectangular glass tanks (76x35x30 cm) and each tank was continuously aerated. Fishmeal protein in control diets was replaced at a rate of 15, 30, 45, and 60% by aquaculture by-product meal protein. Each experimental diet was randomly distributed into triplicate tanks containing catfish fingerlings (n = 15 fingerlings/replicate). The fish were fed in two equal proportions at 0900 and 1700 h for eight weeks. The following water quality parameters, temperature (27.32±0.26ºC); pH

(6.57±0.42), and dissolved oxygen (6.87±0.26 mg/l) were recorded during the period of the experiment.

Blood sampling and assessment: Six (n=6) fish per replicate tank were removed and mildly euthanized with clove oil (100 mg l^{-1}) at the end of the feeding trial for blood sampling. 3.5 ml blood per replicate was obtained by caudal vein piercing using a 1 ml disposable syringe and 25G needle. Approximately, 1 ml blood sample was collected in an ethylene diaminetetraacetic acid (EDTA) treated bottle for haematological examination using an automatic full blood cell counter. The remaining (above 2.5 ml) blood sample was collected in untreated or plain sampling bottles for serum biochemistry after it was allowed to clot at 4°C. After 30 minutes of clotting, the coagulated blood samples were centrifuged at 8,000 rpm for 6 min, and the serum was collected following the procedure of Tomlinson et al. (2013) for onward serum biomarkers assay. The serum total protein was determined using a commercial kit (Randox Laboratories Ltd, U.K), while the bromocresol green method was used to determine albumin value (Doumas et al., 1971; Doumas and Peters Jr, 1997). The differential between the serum total protein and serum albumin formed serum globulin value (Colville, 2002). The method of Toro and Ackermann (1975) was used to determine blood glucose. The procedures of Reitman and Frankel (1957) were used to determine serum alanine aminotransferase (ALT) and serum aspartate aminotransferase (AST). A commercial kit (Randox Laboratories Ltd, U.K) was used to analyze serum creatinine and urea nitrogen by deproteinization and urease-Berhelot colorimetric methods modified by Berthelot-Searcy (Searcy, 1967; Scott, 1979). Low-density lipoproteins (LDL) and high-density lipoproteins (HDL) using kits (BioMed-Cholesterol, Chem. for Lab Technology, Cairo, Egypt) and serum triglyceride were determined by the colourimetric method (Chawla, 2014).

Oxidative stress biomarkers and serum electrolytes: Serum antioxidants, such as catalase (CAT), superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx), thiobarbituric acid and reactive substances (TBARS), were determined spectrophotometrically using commercial kits (Randox^{TM}, United Kingdom), following standard manufacturer's guidelines after homogenization and centrifugation of the serum. Serum electrolytes (Na^+, K^+, $Ca^{2+}$and Cl^{-1}) were obtained using atomic absorption spectrophotometer. These were done at Central Laboratory, Tanke Ilorin.

Proximate analysis: Feed and feed ingredients' proximate composition were assessed with the procedures of AOAC (2010). Dry matter was evaluated after the samples were subjected to 105°C for 24 h in an oven (DHG 9053A, Axiom Medical Ltd, USA). Crude protein content was measured after acid digestion using a protein auto-analyzer (Foss Tecator Kjeltec^{TM} 8400) using a factor of 6.25 to convert the nitrogen into crude protein. Crude lipid was analyzed by the Soxhlet extraction method using Foss Tecator Soxtec^{TM} 8000. The crude fiber was determined using Foss Tecator Fibertec 2010 analyzer. The ash content was obtained by igniting the samples in a furnace (Krupp Widia-Fabrik) at a temperature of 600°C for 4 h. The NFE was calculated using the formula of: NFE = 100(Moisture + Crude protein + Crude Lipid + Ash = Crude Fibre)

Ethical statement: The ethics of animal research as contained in the University of Ilorin's research policy on the use and care of animals were strictly followed.

Statistical Analysis: The data obtained from body indices were expressed as mean ± SE and analyzed using one-way analysis of variance (ANOVA) after the data had passed the Levene test of homogeneity of variance. Duncan multiple range tests were used to separate treatment means where the significant difference (P<0.05) was recorded.

Results

Haematology: The haematological parameters of *C. gariepinus* fed diets containing ABM are presented in Table 2. The primary haematological parameters (haemoglobin, packed cell volume, and red blood cell count) and secondary haematological parameters (Mean Corpuscular Haemoglobin, Mean Corpuscular Volume, and Mean Corpuscular Haemoglobin

Table 2. Haematological parameters of African catfish fed diets containing ABM.

	CTR	D15T	D30T	D45T	D60T
Haemoglobin (g dl^{-1})	11.50±0.65^a	12.50±1.20^a	13.75±0.15^a	11.65±1.25^a	11.35±0.75^a
PCV (%)	39.85±2.05^a	43.4±5.20^a	53.60±4.40^a	40.40±3.60^a	34.35±0.15^a
RBC (10^6 L^{-1})	2.75±0.19^a	3.00±0.40^a	3.89±0.24^a	2.62±0.29^a	2.10±0.35^a
MCH (ρg)	40.50±0.55^a	42.90±0.40^a	41.95±1.65^a	44.45±0.05^a	41.90±1.50^a
MCV (fL)	144.90±2.50^a	146.55±0.05^a	151.55±1.75^a	154.85±2.85^a	140.45±0.35^a
MCHC (g L^{-1})	280.00±1.00^a	293.00±3.00^a	277.50±8.50^a	154.85±5.00^a	298.50±11.50^a

Values (means with standard errors, n=3) within the same row with different superscripts are significantly different (Duncan test; P<0.05) from each other. (Tacon et al., 2009).

Table 3. Serum Biochemistry of African catfish fed diets containing ABM.

	CTR	D15T	D30T	D45T	D60T
Total Protein (mg ml^{-1})	16.18±2.03^a	16.11±0.26^a	12.75±1.60^a	16.65±0.17^a	17.18±0.70^a
Albumin (mg ml^{-1})	13.79±1.66^a	11.01±0.88^a	9.87±0.24^a	11.03±0.11^a	10.82±0.41^a
Globulin (mg ml^{-1})	2.32±0.37^a	5.10±1.14^a	2.88±1.37^a	5.62±0.07^a	6.36±0.29^a
A/G Ratio	5.98±0.23^a	2.31±0.69^a	4.39±2.01^a	1.96±0.01^a	1.70±0.01^a
Urea (mg dl^{-1})	7.55±0.25^a	9.45±1.75^a	11.65±1.65^a	13.55±0.07^a	9.90±0.28^a
Creatinine (mg dl^{-1})	2.19±0.68^b	2.56±0.10^{ab}	1.26±0.17^b	2.60±0.14^{ab}	3.70±0.43^a
HDL-C (mg dl^{-1})	36.60±3.00^a	36.10±2.00^a	32.25±0.05^a	36.70±2.20^a	35.20±0.70^a
LDL-C (mg dl^{-1})	82.55±33.61^a	83.86±4.81^a	82.53±4.46^a	106.77±16.75^a	71.64±17.08^a

Values (means with standard errors, n=3) within the same row with different superscripts are significantly different (Duncan test; P<0.05) from each other.

Table 4. Oxidative stress biomarkers in the blood of African catfish fed diets containing ABM.

	CTR	D15T	D30T	D45T	D60T
SOD (U mg^{-1} Protein)	118.07±19.03^a	154.24±15.99^a	147.63±2.24^a	121.31±31.02^a	153.63±1.61^a
GSH (U mg^{-1} Protein)	156.55±6.28^a	207.10±18.57^a	277.77±9.47^a	174.48±41.26^a	209.81±6.93^a
GPx (U mg^{-1} Protein)	24.82±9.29^a	26.98±3.28^a	24.33±0.59^a	25.44±11.18^a	22.65±0.39^a
CAT (U mg^{-1} Protein)	1932.10±103.48^c	1666.90±62.33^c	3541.4±265^a	2059.5±266.99^{bc}	2674.9±113.71^b
TBARS (U mg^{-1} Protein)	0.26±0.01^a	0.34±0.03^a	0.37±0.02^a	0.29±0.07^a	0.34±0.01^a

Values (means with standard errors, n=3) within the same row with different superscripts are significantly different (P<0.05) from each other. SOD: Superoxide Dismutase; CAT: Catalase; GSH: Glutathione; GPx- Glutathione S-transferase, Thiobarbituric acid reactive substances (TBars).

Concentration) were similar (P>0.05) with control. Figure 1 shows the innate immune response parameters (WBC, its differential, and platelet count) of African catfish fed ABM. The white blood cell count and its differential of the fish group fed ABP meal were numerically higher than control but statistically not significant (P>0.05), except in fish fed D30T that had higher lymphocyte count. The platelet count in all the dietary groups was similar (P>0.05).

Serum biochemistry: There was no significant variation (P>0.05) in some of the serum biochemistry parameters: total protein, albumin, globulin, and albumin/globulin ratio, urea, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) (Table 3). Creatinine values of the D60T-fed group were significantly higher (P<0.05) than all other dietary treatments, including the control. Glucose level was significantly lower (P<0.05) among the ABM-fed groups than in the control group. The cortisol was significantly lower (P<0.05) among the ABP meal-fed groups than the control group except for D45T and D60T (Fig. 2). Lipid function indices (triglycerides and cholesterol levels) of *C. gariepinus* fed diets containing ABM are given in Figure 3. Triglyceride level was similar (P>0.05) to control up to 30% replacement level, while there were no significant variations (P>0.05) in the cholesterol levels of the blood of *C. gariepinus* fed the different dietary treatments.

Oxidative stress biomarkers and serum electrolytes: Except for catalase, there were no significant differences (P>0.05) in other oxidative

Table 5. Serum electrolytes of African catfish fed diets containing ABM.

	CTR	D15T	D30T	D45T	D60T
Calcium (mg dl^{-1})	17.65±2.57^{a}	20.69±0.90^{a}	19.62±0.49^{a}	19.34±1.47^{a}	20.38±0.25^{a}
Chlorine (mEq L^{-1})	17.67±2.05^{a}	18.22±0.90^{a}	13.37±0.09^{a}	±18.81±0.95^{a}	13.95±1.09^{a}
Potassium (mEq L^{-1})	4.01±0.75^{bc}	3.33±0.41^{bc}	2.29±0.08^{c}	6.05±0.44^{ab}	7.05±1.39^{a}
Sodium (mEq L^{-1})	32.18±0.38^{c}	37.05±2.10^{b}	52.05±1.20^{a}	34.13±0.98^{bc}	35.10±0.45^{bc}

Values (means with standard errors, n=3) within the same row with different superscripts are significantly different (P<0.05) from each other.

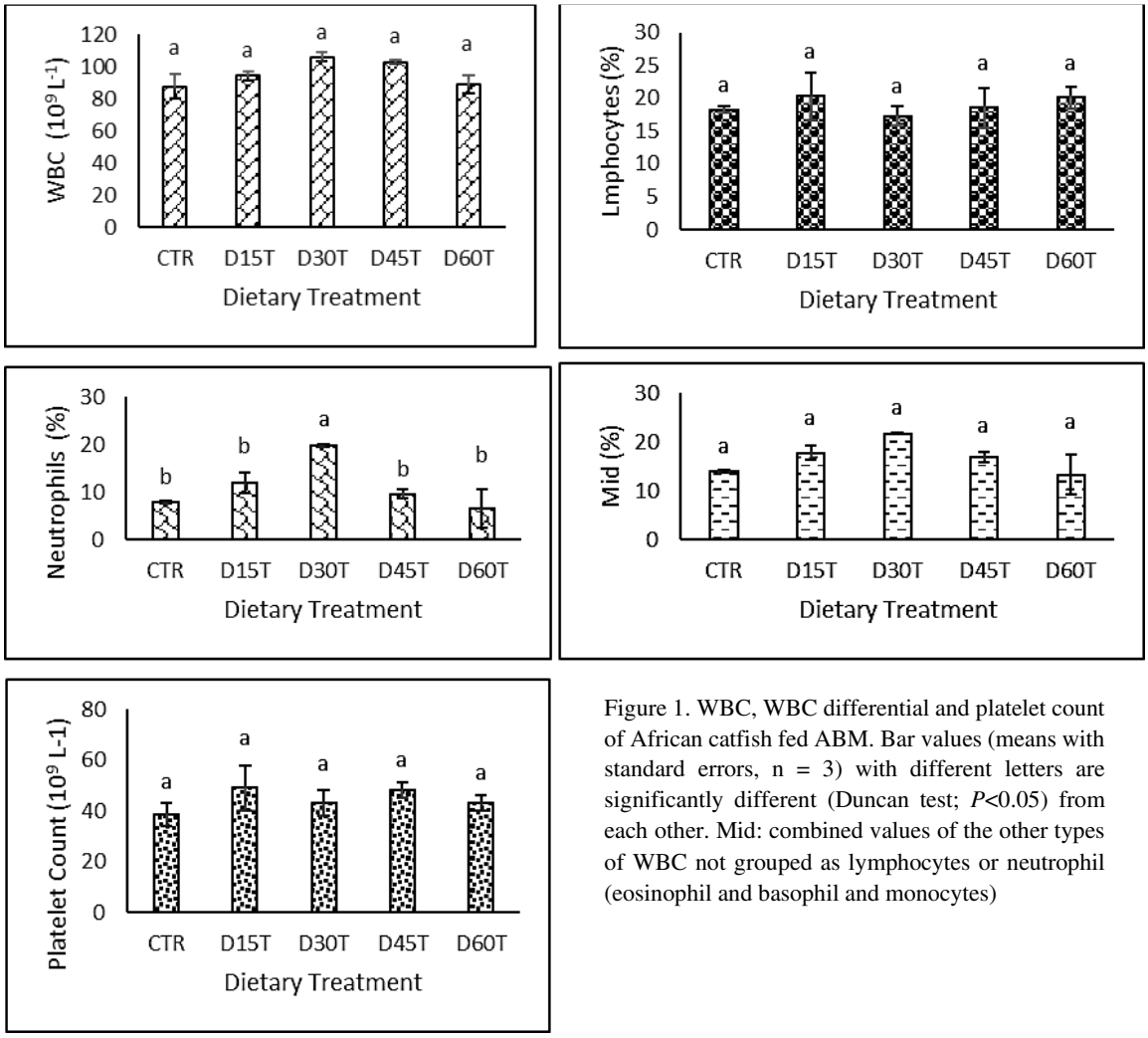

Figure 1. WBC, WBC differential and platelet count of African catfish fed ABM. Bar values (means with standard errors, n = 3) with different letters are significantly different (Duncan test; P<0.05) from each other. Mid: combined values of the other types of WBC not grouped as lymphocytes or neutrophil (eosinophil and basophil and monocytes)

stress biomarkers under study, primarily, SOD, GSH, and GPx (Table 4). Catalase enzyme activities of the fish group fed D30T was higher (P<0.05) than other groups. No significant variations (P>0.05) were recorded in the catalase enzyme of fish fed CTR, D15T, and D45T. T-Bars of the different dietary treatment groups were similar (P>0.05). Some serum electrolytes, such as calcium and chloride ions of the fish fed the different diets, were not significantly different (P>0.05) (Table 5). Serum potassium ions were significantly higher (P<0.05) among D60T-fed group though similar (P>0.05) to the D45T-fed group. Serum sodium ions of the fish group fed D30T was higher (P<0.05) than other groups; the fish group fed D30T was higher (P<0.05) than other fed groups through similarity (P>0.05) exists in serum sodium ion of fish fed CTR, D45T and D60T.

Discussions

This study recorded a lower stress condition occasioned by comparable haematology and the higher antioxidant levels among ABM-fed groups. Hematological parameters are important in evaluating the health status of fish (Fazio et al., 2013; Jimoh et

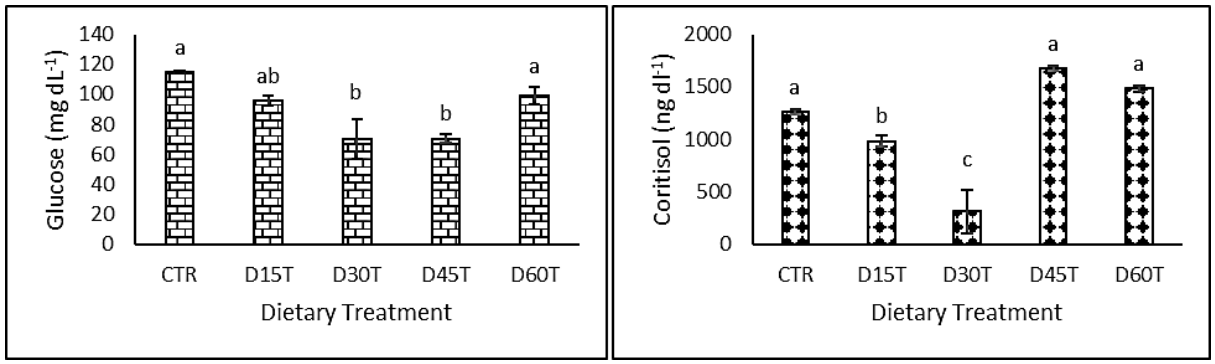

Figure 2. Physiological stress indices (blood glucose and cortisol levels) of *Clarias gariepinus* fed diets containing ABM. Bar values (means with standard errors, n = 3) with different letters are significantly different (Duncan test; $P<0.05$) from each other.

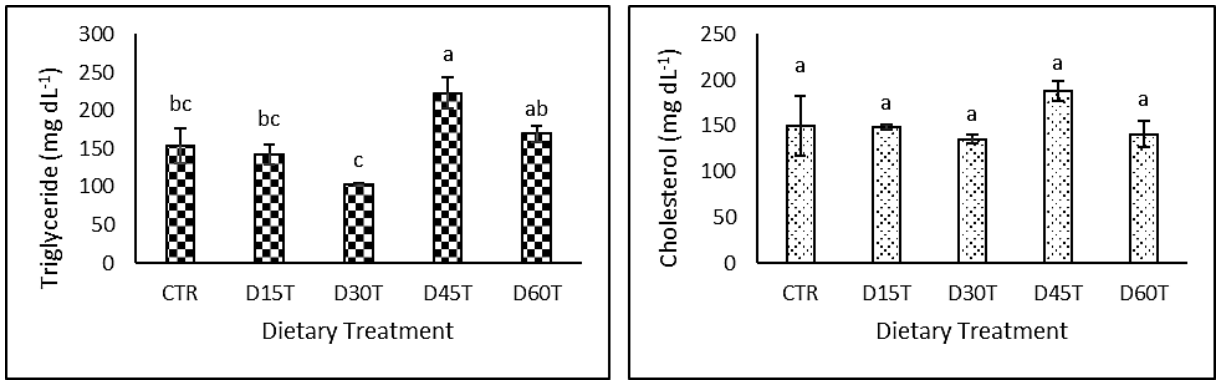

Figure 3. Lipid function indices (triglycerides and cholesterol levels) of *Clarias gariepinus* fed diets containing ABM. Bar values (means with standard errors, n = 3) with different letters are significantly different (Duncan test; $P<0.05$) from each otherr.

al., 2015; Jimoh et al., 2020a, b; Sezgin and Aydın, 2021). Primary haematological parameters (RBC, WBC, Hb, and PCV) recorded in this study show that the fish health was not compromised by feeding ABM. Improved health status of fish is represented by an increase in the level of PCV, RBC, and Hb arising from blood cell component formation (hematopoiesis) and reduced rupture of RBC (hemolysis) in the body system (Clauss et al., 2008; Hoseini et al., 2020b). Hoseini et al. (2020a) reported that haematological parameters, especially RBCs, enhance the oxygenation of tissues which promotes energy expenditure, good health, and growth improvement.

WBC and their differentials, consisting of granulocytes (basophil, neutrophil, and eosinophil) and agranulocytes (lymphocytes and monocytes), are frontline defence systems and constitute innate immune response against invading pathogens in fish (Taheri Mirghaed et al., 2020). A statistically similar elevated level of WBC and their differentials recorded in this study among ABP meal-fed group relative to the control group indicated that the use of ABM in the feed did not have a negative effect on the immune system of the fish similar to what was reported by Yousefi et al. (2012). Thus potentiate a similar capacity to boost innate immunity as the fishmeal. Platelet counts in all the dietary groups were similar, revealing that the fish fed different dietary treatments had a similar immune response. Platelets play a significant role in innate immune and inflammation responses aside from their traditional role in blood clotting (Holinstat, 2017). Plasma globulin improved among ABM-fed fish compared to control though no significant variation existed among the dietary treatment groups. Plasma globulin also plays a diverse role in fish immunity and the antioxidant capacity of fish (Gerwick et al., 2002). Lymphocytes produce immunoglobin (Ig) that helps detect the pathogen (Taheri Mirghaed et al., 2020). Thus, the higher but non-significant level of WBC and their differentials among the fish fed with different dietary treatments potentiates higher but the comparable ability of the

fish to fight against invading pathogen when compared to control. This improves fish health and immune function as evidenced by reduced glucose and cortisol level among the fish group fed ABP meal in this study. Elevated cortisol among the D45T and D60T-fed fish groups could lead to immunosuppression. Cortisol is a stress hormone that activates energy expenditure and immunosupression, thus growth reduction (Dawood et al., 2020; Hoseini et al., 2021; Taheri Mirghaed et al., 2020).

The non-significant levels of oxidative stress biomarkers, primarily antioxidant enzymes (SOD. CAT. GPx and GSH) and lipoperoxidation marker (T-Bars), among the fish, fed the various dietary treatments further showed no stress was recorded by feeding ABM. SOD, GPx and CAT are important components of the antioxidant system (Jimoh et al., 2021), but a higher level of these enzymes in the blood would overwhelm stress (Yilmaz, 2019; Hoseini et al., 2020a). T-Bars being similar among the treatments indicated comparatively no change in the oxidative stress condition of fish fed the different diets. Instead, the antioxidant system was stimulated because the lipid was protected from being oxidized as shown in the elevated levels of T-Bars among the ABM-fed fish. The increased level of T-Bars was consequently counteracted by the increased antioxidant enzyme activities to reduce the negative effect of oxidative stress. This is consistent with the reports of Hoseini et al. (2019) and Rajabiesterabadi et al. (2020). Antioxidants offer profound protection to RBC membrane lipid from being oxidized, guiding against oxidative stress conditions which cause lower RBC destruction and hemolysis (Yang et al., 2013; Gao et al., 2016; Hoseini et al., 2020a). Lipid function indices, such as triglycerides and cholesterol in this study, appeared to have a similar trend to the report of Saleh et al. (2020) on European seabass (*Dicentrarchus labrax*) fed bioconverted fish waste.

The serum ionic composition of fish plays important role in the maintenance of osmotic potentials and the normal physiological functioning of blood cells (Edori et al., 2013). Hence, they could be used as indices of assessing stress conditions in fish because of their osmoregulatory functions (Shui et al., 2018). Mayer et al. (1992) reported serum Na^+, Ca^{2+}, K^+, and Cl^- are important for the regulation of internal homeostasis because they are indispensable elements of cellular metabolism (Karthikeyan et al., 2006). The statistical similarity that existed in serum $Ca^{2+}$and Cl^- among dietary treatment groups further suggested that feeding ABM did not impact negatively on the blood cells' permeability property, hence no stress condition among the dietary groups. Ca^{2+}, at a significantly elevated level, could damage the permeability of the blood cell membrane (Hoar, 1983; Edori et al., 2013). Thus, blood cell integrity was protected as the serum electrolytes were similar to control. To the best of our knowledge, work on the effect of dietary treatment on serum electrolytes is little investigated. This report constitutes the first mention of it on fed *C. gariepinus*. A lot of investigations abound on the impact of salinity gradient or toxic constituents on the serum or tissue electrolytes of fish (Wood, 2011; Stewart et al., 2016; Shui et al., 2018). Serum Na^+ and K^+ moved between blood cells and plasma by active transport to maintain the blood volume in the body and enhance the body's physiological functioning. K^+ was statistically similar to control up to 45% replacement level by ABM in this study. So also, the Serum Na^+ of the control group was the lowest but shared statistical similarity with the fish group fed D45T and D60T. Fish attains equilibrium by the balance between Na^+ and K^+ as they participate in the transport of K^++ Na^+-ATPase activities (Towle and Mangum, 1985; Edori et al., 2013). The normal range of serum K^+ maintains the integrity of the central nervous system (Adedeji, 2010). Significant reduction of serum Na^+ could negatively impact the iso-osmolarity condition of the blood cell resulting in stress (Gabriel et al., 2009). Hence, this could be a pointer to osmoregulatory difficulties (Laiz-Carrión et al., 2005a, b). Since statistical similarity exists with control in the different constituents of serum electrolytes understudy, no stress condition among the dietary groups could be further deduced.

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