HAEMATOLOGY AND HISTOPATHOLOGY OF *CLARIAS GARIEPINUS* FED *ASPERGILLUS BRASILIENSIS* FERMENTED PALM KERNEL SLUDGE IN REPLACEMENT OF MAIZE

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Abstract

This study assessed the haematology and histopathology of *Clarias gariepinus* fed *Aspergillus brasiliensis* fermented Palm Kernel Sludge (AbfPKS) in replacement of maize. The AbfPKS was used to replace maize at 0%, 25%, 50%, 75% and 100%. Polyzyme (commercial enzyme) was added to the sixth treatment containing unfermented PKS. The experiment was a completely randomised design and data obtained was analysed using ANOVA at $\alpha_{0.05}$. Means observed were separated using Duncan's Multiple Range Test (DMRT). The packed cell volume (PCV) of fish fed AbfPKS ranged between 16.33±0.58% and 29.00±1.00%. Highest haemoglobin (Hb) was recorded in fish fed diet 5Ab (9.63±0.23gm%) while least Hb was recorded in fish fed diet 2Ab (5.30±0.17gm%). The WBC varied from 6.27±1.40 (x $10^{9/L}$) to 10.53±0.83 (x $10^{9/L}$). Platelets ranged between 4.00±0.00 (x $10^{9/L}$) to 8.67±1.15 (x $10^{9/L}$). The ESR significantly ($p<0.05$) increased as inclusion of AbfPKS increased in the diet of fish. Histopathological observations on liver, kidney and intestine of fish fed AbfPKS showed that livers of experimental fish had hepatocellular degeneration and necrosis of hepatocytes. Fish fed dietary treatments did not have any observable lesion in their intestines. Fish fed dietary treatments had necrosis, tubular epithelial degeneration with atrophy of tubules present in their kidneys. Pathological signs observed in liver of experimental fish were not as a result of feeding on AbfPKS because these signs were also observed in fish not fed AbfPKS. Feeding on AbfPKS did not cause any observable lesion in the intestine of all experimental fish.

Keywords: *Aspergillus***,** ferment, signs, maize

Introduction

The competition by Nigerian livestock feed industry with humans for energy feed resources such as maize, wheat and sorghum has necessitated the search for alternatives through the improvement of non-conventional feed ingredients and agro-wastes (Iyayi, 2004; Tarhazadesh, 2010). One of such improvement efforts is the solid state fermentation of oil palm by-products by Omoikhoje, *et al.,* (2014) and Folorunso, (2018) to compound livestock feeds. The abundance of palm products was a major reason that stimulated interest in exploring its usage in fish diets (Ng, 2003). But it is noteworthy that feed of plant origin have a great influence on histological changes in liver and intestine of fish (Rašković *et al*., 2011). Thus, this study was aimed at assessing the haematology and histopathology of *Clarias gariepinus* fed *Aspergillus brasiliensis* fermented Palm Kernel Sludge (AbfPKS) in replacement of maize.

Materials and Methods

The average weight, Standard Length (SL) and Total Length (TL) of fish used was (6.03±0.65g), (8.26±0.26cm) and (9.60±0.35cm) respectively. Each treatment contained 60 pieces of fish while 20 were per replicate. The fish were fed at 5% body weight per day with their feeding rate adjusted every forthnight according to the new body weight. They were reared for a period of 16 weeks. Six (6) isonitrogenous and isocalorific diets were formulated to contain 40% crude protein as recommended by Faturoti (2000). Production of AbfPKS was as described by Folorunso *et al.,* (2018). Gross composition of diets used contained Maize**:**AbfPKS combination (18.49%), wheat offal (4.1%), Soy cake (48.22%), fishmeal (25.11%), DCP (2%), salt (0.3%), vitamin premix (0.25%), lysine (0.1%), methionine (0.1%) and starch (1.5%). Diet 1Ab contained 100% maize. Diet 2Ab contained 75% maize and 25% AbfPKS. Diet 3Ab

contained 50% maize and 50% AbfPKS. Diet 4Ab had 25% maize and 75% AbfPKS. Diet 5Ab did not contain maize and maize was replaced with 100% AbfPKS. Diet 6Ab contained unfermented PKS plus Polyzyme. The diets were pelletised through a 2mm die, dried, kept in polythene bags and stored in a cool dry place before use. At the end of the feeding trial, fish were fasted for 24 hours prior to the collection of blood samples from the caudal peduncle. Blood from two (2) pieces of fish from each replicate was pooled to get enough blood for analysis. Haematocrit (PCV %), Haemoglobin concentration (Hb g dL^{-1}), Red Blood Cells count (RBCs) and white blood cells (WBC) were determined using standard formulae while erythrocyte sedimentation rate (ESR mm h^{-1}) was determined as described by WHO (2003). Also, a fish was selected from each replicate. The head kidney, liver and intestine were harvested and processed using standard laboratory methods. The experiment was a completely randomised design and data obtained was analysed using ANOVA at $\alpha_{0.05}$. Means observed were separated using Duncan's Multiple Range Test (DMRT).

Results and Discussion

Haematological parameters of fish fed AbfPKS are presented in Table 1. The PCV of fish fed AbfPKS ranged between 16.33±0.58% (diet 2Ab) and 29.00±1.00% (diet 5Ab). Highest Hb was recorded in fish fed diet 5Ab (9.63±0.23gm%) while least Hb was recorded in fish fed diet 2Ab (5.30±0.17gm%). Fish fed diet 3Ab had highest RBC (5.22±0.94) x $10^{12/L}$ which was similar to RBC of fish fed diet 6Ab (4.37±0.39) x $10^{12/L}$ but was significantly different ($p<0.05$) to RBC of fish fed diets 1Ab, 2Ab, 4Ab and 5Ab. The WBC varied from 6.27±1.40 (x $10^{9/L}$) (diet 6Ab) to 10.53±0.83 (x $10^{9/L}$) (diet 2Ab). Platelets ranged between 4.00±0.00 (x $10^{9/L}$) diet 2Ab to 8.67±1.15 (x $10^{9/L}$) in diet 5Ab while Lymphocytes varied from 22.33±0.58% (diet 6Ab) to 45.67±0.58% (diet 4Ab). Neutrophils ranged from 53.33±0.58% (diet 4Ab) to 76.67±0.58% (diet 6Ab). The ESR significantly ($p<0.05$) increased as inclusion of AbfPKS increased in the diet of fish. In this study, fish fed diet 2Ab containing the least amount of AbfPKS had lower PCV than fish fed the control and other diets containing higher amount of AbfPKS. The non reduction in PCV and RBC of fish fed high replacement levels of maize with AbfPKS shows that the experimental fish did not experience anaemia and are of good health status and that the anaemic condition observed in diet 2Ab may not necessarily be as a result of feeding on AbfPKS. Also, inclusion of AbfPKS in fish diets enhanced RBC and haemoglobin values of the experimental fish. This may be due to more minerals and vitamins (Lawal *et al.*, 2015) made available by inclusion of AbfPKS. The non significant difference in WBC of fish fed AbfPKS is an indication that immunity was not compromised. Because, when WBC is elevated, this indicates immune system of animals is responding to a factor which can be a disease or environmental challenge (Ahamefule *et al*., 2008). The ESR of experimental fish significantly increased ($p<0.05$) with replacement of maize with AbfPKS while the fish whose diet contained 100% maize had the lowest ESR. This implies that a higher inclusion of AbfPKS in the diets elicited an increase in the ESR.

Histopathological observations on liver, kidney and intestine of fish fed AbfPKS are presented in Table 2. The histological integrity of the organs (liver, intestine and kidney) is fundamental to the well being of the fish. Cellular degeneration and necrosis observed in liver of fish fed AbfPKS as well as those that were fed control diet implies that AbfPKS was not responsible for the pathological signs observed in the liver. Feeding AbfPKS did not cause any observable lesion in the intestine of all experimental fish. Tubular epitheliar degeneration and necrosis was observed in kidney of fish fed varying amounts of AbfPKS. While fish that were not fed AbfPKS did not have such signs in their kidney.

Conclusion

Pathological signs observed in liver of experimental fish were not as a result of feeding on AbfPKS because these signs were also observed in fish not fed AbfPKS. Feeding on AbfPKS did not cause any observable lesion in the intestine of all experimental fish.

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Table 1. Haematological parameters of *Clarias gariepinus* fed AbfPKS in replacement of maize

	PCV %	HB gm%	RBC x $10^{12/L}$	WBC x $10^{9/L}$	Platelets x $10^{9/L}$	Lymphocytes %
Diet 1Ab	21.33±0.58^{c}	7.00±0.17^{c}	3.37±0.78^{b}	7.73±1.67^{b}	6.00±0.00^{b}	30.67±0.58^{c}
Diet 2Ab	16.33±0.58^{d}	5.30±0.17^{d}	3.50±0.41^{b}	10.53±0.83^{a}	4.00±0.00^{c}	37.67±0.58^{b}
Diet 3Ab	28.33±1.15^{ab}	9.30±0.35^{ab}	5.22±0.94^{a}	7.60±1.11^{b}	8.00±0.00^{a}	23.67±0.58^{d}
Diet 4Ab	27.33±0.58^{b}	9.10±0.35^{b}	3.49±0.54^{b}	6.80±1.83^{b}	8.00±0.00^{a}	45.67±0.58^{a}
Diet 5Ab	29.00±1.00^{a}	9.63±0.23^{a}	3.43±0.40^{b}	8.40±1.74^{ab}	8.67±1.15^{a}	30.33±0.58^{c}
Diet 6Ab	16.33±1.15^{d}	5.30±0.35^{d}	4.37±0.39^{ab}	6.27±1.40^{b}	4.00±0.00^{c}	22.33±0.58^{e}
RV	36.00±9.04	6.04±25.57	2.88±0.70	41.1±0.70	NA	34.12±17.51

Key : PCV = Packed Cell Volume, HB = Haemoglobbin, RBC = Red Blood Cells, WBC = White Blood Cells, ESR = Erythrocyte Sedimentation Rate. Values presented are means of six (6) readings. Triplicate samples of all fish samples were each done in duplicates. (n=6). Values followed by the same letter within the same column are not significantly different at 5% level of significance (DMRT). ($p<0.05$). RV= Reference value as reported by Erhunmwunse and Ainerua (2013). NA= Reference value for such parameter was not available.

Table 2 Histopathology of *Clarias gariepinus* organs when AbfPKS was used to replace maize in their diet

	Liver	Intestine	Kidney
Diet 1Ab	Moderate diffuse hepatocellular degeneration (swelling)	No observable lesion	No observable lesion
Diet 2Ab	Hepatocellular swelling and cholestasis	No observable lesion	Patchy tubular epithelia coagul
Diet 3Ab	Diffuse degeneration and necrosis of hepatocytes	No observable lesion	Marked diffuse tubular epitheli with atrophy of tubules
Diet 4Ab	Moderate cloudy hepatocellular degeneration (swelling)	No observable lesion	Moderate tubular atrophy and r cells
Diet 5Ab	Cloudy degeneration of hepatocytes	No observable lesion	Tubular epithelia coagulation r
Diet 6Ab	Degeneration and necrosis of hepatocytes	No observable lesion	No observable lesion