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Research Article

Hematological, Histopathological and Internal Organs Response of African Catfish Clarias Gariepinus to Diets Containing Auto-Detoxified Mixtures of Jatropha Kernel Cake with Bovine Blood in Replacement of Fishmeal

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Abstract

Haematological, Histopathological, and Internal organs response of African catfish (Clarias gariepinus) fingerlings, were used as selection criteria for three auto-detoxified mixtures of Jatropha kernel cake with bovine blood (ADMJKC/bb) coded, Y3, Z2, and Z4. Each of the three test ingredients was evaluated at a 30 % and a 50% replacement level with fishmeal. Thus with a control, the ingredients were compared in seven iso-nitrogenous and iso-caloric diets (control, Y330, Y350, Z230, Z250, Z430, and Z450). The control significantly ($P<0.05$) had higher haemoglobin level, packed cell volume (PCV) and lower white blood cell count (WBC) than the ADMJKC/bb test ingredients. Similarly, increasing the ADMJKC/bb from 30% to 50% of reduced the PCV and increased WBC. However, the PCV and WBC in Z430 were significantly ($P<0.05$) different from their Z230 and Y330. counterparts. Histopathology and internal organ response followed similar trends as haematology. This lead to the conclusion that, the Y3 and Z2 ADMJKC/bb ingredients were better detoxified than the Z4 ingredient. However, Y3 and Z2 should not replace more than 30% of fishmeal diets for *C. gariepinus* fingerings. Therefore, there is a need to continue development of the Y3 and Z2 ingredients for *C. gariepinus* and other farm animals.

Keywords: auto-detoxifed; Jatropha-kernel-cake; bovine-blood; haematology; histopathology; spleen-somatic-index; Clarias gariepinus

Abbreviations

ADMJKC/bb: auto-detoxified mixtures Jatropha kernel cake and bovine blood; RBC: Red blood cell count; PCV: packed cell volume; WBC: white blood cell count; Hb: haemoglobin concentration; ALT: alanine aminotransferase; ALP alkaline phosphatase;. RIL: Relative intestinal length; ISI: intestinal somatic index; HIS: hepatosomatic index; SSI: spleen somatic index; Y3: Jatropha kernel cake and bovine blood, mixed at a ratio of 2:1. Heated, spread dried, remoisten to 66% dry matter; Z2: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried without remoistening; Z4: Jatropha kernel cake and bovine blood, mixed at a ratio of 3:1. Unheated, spread dried, Remoistened to 66% dry matter.

Introduction

The use of haematology, histopathology, and internal organs as identifiers of the state of nutrition and health in Clarias gariepinus (Temitope et al., 2020) as well as other fish (Harkrishan et al., 2020) and farm animals like chicken (Hussein et al.,2020) abound in the literature. However, experiments accessing the potential danger of substances an animal is exposed to may not go through the entire lifecycle of the animal. This increases the need for facts on the physiological and biochemical benchmarks, which ascertain that, the danger of toxicity has been wholly or partially, eliminated irrespective growth and economic data indicators. For example, jatropha kernel cake (JKC), which is the main component of auto-detoxified mixtures of jatropha kernel cake, with bovine blood (ADMJKC/bb), is toxic to humans and animals because of its phorbol esters and other anti-nutrients (trypsin inhibitors, lectins and phytate), and therefore requires detoxification before use (Ewane et al., 2017). Mixing it with bovine blood and processing the mixture through an auto-detoxification process reduces its toxicity as proven in in-vitro tests with brine shrimp, *Artemia salina* (Ewane et al.,2021a) and *in-vivo* tests with *C. gariepinus* (Ewane et al., 2021b). However, it is not clear how the physiological and biochemical parameters of farmed animals like *C. gariepinus* are affected in an *in-vivo* feeding trial. This study was therefore performed to generate additional knowledge with respect to haematology, histopathology, and internal organs of *C. gariepinus* fingerlings. Such knowledge will aid in selecting one of three ADMJKC/bb test ingredients produced and reported elsewhere.

Materials and Methods

Experimental Ingredients and Diets

Auto-detoxified mixtures of Jatropha kernel cake and bovine blood (ADMJKC/bb) were produced as described in Ewane et al. (2021a), and the three top ingredients recommended (Table 1) were tested in iso-caloric and iso-nitrogenous diets at 30% and 50% replacement levels for fishmeal. These test ingredients were coded as Y3, Z2 and Z4).

Code of Recommended ADMJKC/bb test ingredients	***Description (Preparation protocol)***
Y3	Jatropha Kernel cake (JKC) mixed with bovine blood (bb) at a ratio of 2:1. Heated, spread dried, and remoistened daily to 66%DM
Z2	Jatropha Kernel cake (JKC) mixed with bovine blood (bb) at a ratio of 3:1. Unheated, spread dried without remoistening
Z4	Jatropha Kernel cake (JKC) mixed with bovine blood (bb) at a ratio of 3:1. Unheated, spread dried, and remoistened daily to 66% DM

Table 1: Description of Auto-detoxified mixtures of Jatropha kernel cake and bovine blood (ADMJKC/bb) test ingredients.

Seven iso-nitrogenous and iso-calorifc experimental diets (Table 2) were prepared as described in Ewane et al. (2021b).

Treatments			Ingredients									
Fish meal	ADMJKC/bb*	Code	Maize	Soybean cake	ADMJKC/bb	Blood meal	Fish meal	Palm oil	Bone meal	$T1O_2$	Premix **	Total
100%	Control	-	36	0	0	3	50	5	2	1	3	100
70%	30% Y3	$Y3_{30}$	25	11	15	3	35	5	2	1	3	100
50%	50% Y3	$Y3_{50}$	16.2	19.8	25	3	25	5	2	1	3	100
70%	30% Z2	$Z2_{30}$	32	4	15	3	35	5	2	1	3	100
50%	50% Z2	$Z2_{50}$	29	7	25	3	25	5	2	1	3	100
70%	30% Z4	$Z4_{30}$	26.3	9.7	15	3	35	5	2	1	3	100
50%	50% Z4	$Z4_{50}$	18.5	17.5	25	3	25	5	2	1	3	100

* Auto-detoxified mix of Jatropha kernel cake and bovine blood.

** Premix: Composed (mg vitamin and mineral/kg premix): vitamin A 4,800,000 IU, vitamin D_3 800,000 IU, vitamin E4800 mg, vitamin K 800 mg, thiamine 600 mg, riboflavin 2800 mg, vitamin B_3 4800 mg, pyridoxine 600 mg, vitamin B_{12} 4 mg, folic acid 200 mg, cobalt 160 mg, copper 1200 mg, iron 9000 mg, iodine 480 mg, magnesium 2730 mg, manganese 28000 mg, zinc 20000 mg

Table 2: Composition of experimental diets.

Experimental system and animals

A total of 315 fingerlings of African Catfish (Clarias gariepinus) with an average weight of 2.90±04 were allotted to seven treatments, each treatment having three replicates. These were distributed in 21 aquaria, each having a capacity of 35 l. Water quality parameters were monitored daily to stay within optimum range (Temperature 23-26oC, pH 7-8, dissolved oxygen: 5.0 to 6.8 mg l-1, conductance 65.6 -107µhom/cm3, total NH3 0.1- 0.2 mg l-1, nitrite 0.02 -0.08 mg l-1 and nitrate 1-3 mg l-1). The fish were fed 5% of their weekly body weight daily.

Internal organs

Relative intestinal length (RIL), intestinal somatic index (ISI), hepatosomatic index (HSI), and spleen somatic index (SSI) were evaluated. The RIL, ISI, HSI, and SSI were calculated as follows:

RIL (mm g-1) = Intestine length (mm)/ body mass (g).
ISI (%) = (intestine mass (g)/ body mass (g)) × 100.
HSI (%) = (liver mass (g)/ body mass (g)) × 100.
ISI (%) = (Spleen mass (g)/ body mass (g)) × 100.

Blood

The following blood parameters were evaluated: Red blood cell count (RBC), packed cell volume (PCV), white blood cell count (WBC), haemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), creatinine, alanine aminotransferase (ALT), and alkaline phosphatase (ALP).

The procedures of Chronolab (2016) were used in the analysis of Creatinine, ALT and ALP. Red blood cells (RBC) and white blood cells (WBC) were counted using Neubauer's improved haemocytometer. Haemoglobin (Hb) was evaluated by the cyanomethaemoglobin method. Packed cell volume (PCV) was assessed by use of a microhaematocrit. Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were estimated using the calculations of Baker and Silverton (1982)

Histopathological studies

At the end of the feeding trial, fish were prepared for histopathological studies as described by Bancroft and Stevens (1977). The

liver, muscle and intestinal samples from the various groups of experimental fish were collected for histopathology. They were fixed in methanol/acetic acid for 48 h before dehydration in ethanol. After that, the tissues were cleared for 12 hours in chloroform. Then they were infiltrated and embedded in molten paraffin wax. After this, the blocks were clipped and split at 5-6 microns. The sectors were deparaffinised using xylene. They were then immersed in water and afterwards stained with haematoxylin and eosin. Selected sections were captured onto a computer with Moticam 2001 camera attached to a Moticam microscope (Moticam products, London, UK).

Statistical analysis

One-way analysis of variance (ANOVA) was used to analyse the data. Any significant differences between means were tested using Duncan's multiple range tests at 5% level of probability. The statistical package for social sciences (SPSS) version 22 (IBM Corp. Released 2013) software was used.

Results

Haematological response to diets

Table 3 shows the haematological response of *C. gariepinus* fingerlings to ADMJKC/bb diets, while Table 4 shows the response of liver enzymes-Alkaline phosphatase (ALP, U/l), and Alanine transaminase (ALT, U/l)-, and creatinine (Creat, µmol/l), The haemoglobin level was signifcantly (P<0.05) higher for the control fish than the fishes fed the test ingredients. Within the test ingredients, the haemoglobin content of Z230 was higher (P<0.05) than that of the Z430, Z450 and Y350 ingredients. The control also had higher (P<0.05) packed cell volume (PCV) than the ADMJKC/bb diets. Similarly, increasing the ADMJKC/bb from a 30% to a 50 %, reduced (P<0.05) the PCV. In addition, the PCV in Z430 was significantly (P<0.05) lower than Z230 and Y330. The control had a lower (P<0.05) white blood cell count (WBC) compared to the ADMJKC/bb ingredients. The WBC response to an increasing level of fishmeal replacement from 30% to 50% was significant (P<0.05) for all test ingredients. Alkaline phosphatase (ALP) levels were comparable (P>0.05) for the control and the 30% of fishmeal replacement. However, the 30% ADMJKC/bb diets had significantly (P<0.05) lower levels of ALP compared to their 50% counterparts. The ALT levels were significantly (P<0.05) lower for the control compared to the ADMJKC/bb diets. The ALT increased significantly (P<0.05) with increasing the level of test ingredient from 30% to 50%, for Y3 and Z2. However, the difference between Z430 and Z450 was not significant (P>0.05). Blood creatinine levels were statistically similar for all treatments.

Treatment	Hb (g/100ml)	RBC ($x10^6$/µl)	PCV (%)	RBC Indices			WBC ($x10^3$/µl)
				MCV (fl)	MCH (pg)	MCHC (%1g/100ml)	
Control	8.8^{d}±0.06	3.05±1.03	26.43^{d}±0.33	88.99^{c}±0.68	34.68±15.02	31.16^{ab}±1.70	9.20^{a}±0.06
$Y3_{30}$	6.73^{bc}±0.27	2.60±0.91	25.70^{c}±0.28	77.40^{b}±5.81	27.79±11.61	30.89^{ab}±2.24	11.17^{c}±0.12
$Y3_{50}$	6.33^{ab}±0.34	2.25±1.08	24.25^{a}±0.35	72.91^{a}±1.60	25.18±10.21	32.23^{ab}±0.17	11.80^{d}±0.07
$Z2_{30}$	6.86^{c}±0.08	2.62±0.71	25.33^{c}±0.32	73.47^{ab}±1.21	27.18±11.51	30.43^{a}±0.37	10.73^{b}±0.52
$Z2_{50}$	6.70^{bc}±0.14	2.31±0.85	24.13^{a}±0.09	71.7^{a}±0.68	25.24±10.84	32.68^{bc}±0.41	11.30^{c}±0.12
$Z4_{30}$	6.43^{ab}±0.14	2.57±1.13	24.76^{b}±0.22	72.57^{a}±1.21	27.05±11.54	32.66^{bc}±0.52	11.77^{d}±0.29
$Z4_{50}$	6.23^{a}±0.18	2.18±0.47	24.03^{a}±0.10	70.90^{a}±0.38	25.21±6.30	33.15^{c}±0.33	12.43^{e}±0.10
SEM	0.19	0.18	0.19	1.38	2.26	0.30	0.22

Values are mean (n = 3) ± standard deviations.

Means in the same column with different superscript are significantly (P < 0.05) different.

Hb =Haemoglobin; RBC= Red blood cells; PCV=Packed cell volume; MCV= Mean cell volume; MCH= Mean corpuscular haemoglobin.

MCHC= Mean corpuscular haemoglobin concentration and WBC= white blood cell.

SEM = standard error of the mean.

Table 3: Haematological response of *Clarias gariepinus* fingerlings fed ADMJKC/bb diets for 8 weeks.

*Treatment**	*ALP (U/l)*	*ALT (U/l)*	*Creatinine (µmol/l)*
Control	1.04^{a}±0.28	1.03^{a}±0.68	1.08±0.55
$Y3_{30}$	0.66^{a}±0.29	2.87^{b}±0.70	1.07±0.22
$Y3_{50}$	36.51^{b}±2.15	6.04^{c}±0.87	0.8±0.32
$Z2_{30}$	0.53^{a}±0.29	3.10^{b}±0.77	0.97±0.14
$Z2_{50}$	36.21^{b}±1.14	6.73^{c}±0.74	0.6±0.29
$Z4_{30}$	0.63^{a}±0.36	5.50^{c}±1.06	0.87±0.32
$Z4_{50}$	39.35^{c}±1.13	6.60^{c}±1.53	0.71±0.34
SEM	4.06	.49	.07

Values are means (n = 3) ± standard deviation.

Means in the same column with different superscript are significantly (P < 0.05) different.

1 U = 16.66 nKat/l; nKat = Amount of glandular kallikrein which cleaves 0.005 mmol of substrate per minute.

SEM = standard error of the mean.

Table 4: Alkaline phosphatase (ALP, U/l) Alanine transaminase (ALT, U/l) and creatinine (Creat, µmol/l) in blood of *Clarias gariepinus* fingerlings fed ADMJKC/bb diets for 8 weeks.

Internal organs - intestines, liver and spleen-response to ADMJKC/bb diets

Table 5 shows relative intestinal length (RIL), intestinal somatic index (ISI), hepatic somatic index (HSI), and spleen somatic index (SSI) of *C. gariepinus* fingerlings fed ADMJKC/bb diets. Fishes from the control and ADMJKC/bb diets fed at a 30% inclusion did not differ significantly (P>0.05) in relative intestinal length (RIL). The Y330 and Y350 also did not differ significantly (P>0.05) in RIL. However, Z250 and Z450 considerably (P<0.05) had higher RIL than their Z230 and Z430 counterparts. Similarly, the Z450 and Z250 considerably (P<0.05) induced higher ISI than the control. The control had similar (P>0.05) ISI with Y330, Y350, Z230, and Z430. However, there was no significant difference (P>0.05) among the ADMJKC/bb diets in the ISI. The Y350 ingredient induced the smallest spleen somatic index (SSI). This was not different (P<0.05) from Z250 and Z450. The SSI of Y350 fed fishes, however, differed significantly (P<0.05) from the control and the other 30% fed fishes (Y330, Z230, and Z430). Conversely, the Z230 ingredient induced the largest SSI, which did not significantly differ (P>0.05) from the control, Y330, Z430, and Z450 ingredients. All treatments, including the control, were statistically similar (P>0.05) in HIS.

*Treatment**	*RIL*	*ISI*	*HIS*	*SSI*
Control	132.43^{a}±22.68	3.44^{a}±1.05	1.77±0.29	0.050^{bc}±0.008
$Y3_{30}$	201.81^{ab}±59.54	6.30^{ab}±3.34	1.72±1.07	0.053^{bc}±0.023
$Y3_{50}$	279.17^{bc}±59.92	4.57^{ab}±0.80	1.27±0.45	0.025^{a}±0.008
$Z2_{30}$	208.26^{ab}±30.73	5.61^{ab}±0.60	1.65±0.22	0.062^{c}±0.010
$Z2_{50}$	433.98^{d}±43.50	6.52^{b}±1.07	1.20±0.85	0.039^{ab}±0.001
$Z4_{30}$	240.84^{ab}±51.13	4.9^{ab}±0.81	1.23±0.25	0.052^{bc}±0.008
$Z4_{50}$	331.98^{cd}±107.41	7.08^{b}±1.24	1.14±0.44	0.042^{abc}±0.011
SEM	23.14	0.38	0.12	0.00

Values are means (n = 3) ± Standard deviation.

Means in the same column with different superscript are significantly (P < 0.05) different.

SEM = standard error of the mean.

Table 5: Relative intestinal length (RIL), Intestinal somatic index (ISI), Hepatic somatic index (HSI) and Spleen somatic index (SSI) of *C. gariepinus* fingerlings fed ADMJKC/bb diets for 8 weeks.

Histopathological response to ADMJKC/bb diets

Table 6 and Figures 1 to 3 indicate the histopathological response to ADMJKC/bb diets in the liver, muscle and intestines. The liver sections (Figure 1) from the control group indicted normal histological features typical of fishes. These include the unorganized cords of hepatocytes, radiating from the central vein, separated by sinusoids and unapparent portal triads. Pale cytoplasm, which is indicative of glycogen storage in fishes, was also observed in the liver sections. This should not be confused with hepatic lipidosis/steatosis. Interestingly, pale cytoplasm, was not observed in the ADMJKC/bb fed groups. The liver sections of the ADMJKC/bb fed groups did not present remarkable pathological changes except Z250, Z430 and Z450, which showed focal areas of hepatocyte necrosis and mononuclear cell infiltration. Conversely, Y330, Y350, and Z230 were normal.

Figure 2 shows photomicrographs of the muscle. The control, Y330, Y350, and Z230 groups revealed normal histo-architecture typical of skeletal muscles in fish. This includes bundles of very long, cylindrical, multinucleated cells that show cross striations. However, Z250, Z430, and Z450 disclosed mild to moderate muscle degeneration and necrosis with mononuclear cell infiltration in between the myofibres.

Figure 3 shows photomicrographs of the intestine. The control, Y330 and Z230 showed normal histology. This is indicated in the various tissue layers. The innermost mucosa comprises the columnar epithelial lining, with varying degrees of goblet cells, and an underlying lamina propria, rich in blood vessels, lymphocytes, and lymphatics. This is followed by the submucosa, comprising irregular dense connective tissues, blood vessels and lymphatics. The third layer, called the muscularis, comprises of smooth muscle cells, with distinct orientation. Finally, the outermost serosa is the thin layer of fibrous connective tissue covering the intestine. It is not typical in these photomicrographs. There were visible pathological alterations in the intestine of Z250, Z430, and Z450. These included necrosis of enterocytes and sloughing/denudation of the tips of the villus, leading to a reduction in their sizes. However, there was mild oedema of the sub-mucosa and lamina propria in Y350.

Pathology	***Intensity of Pathology***						
	Control	***$Y3_{30}$***	***$Y3_{50}$***	***$Z2_{30}$***	***$Z2_{50}$***	***$Z4_{30}$***	***$Z4_{50}$***
LIVER							
Necrosis	-	+	+	+	++	++	+++
Decreased glycogen	-	++	++	++	++	++	++
Sinusoidal distension	-	+	+	+	++	++	++
Mononuclear cell infiltrates	-	-	-	-	+	++	++
Increased glycogen	++	+	+	+	+	+	+
Vacuolations	+	+	+	+	+	+	+
MUSCLE							
Necrosis	-	+	+	+	++	+++	+++
Degeneration	-	+	+	+	++	++	++
Mononuclear cells infiltration		+	+	+	++	+++	+++
INTESTINE							
Increased goblet cell size	+	+	+	-	-	+	+
Inflammatory cells infiltration	+	+	++	+	+++	+++	+++
Vilus atrophy	-	+	+	+	+++	+++	++
Oedema of lamina propria	-	+	++	+	+	+	+

Absent: -, Mild: +, Moderate: ++, Severe: +++, Very severe: ++++

Table 6: Summary of histopathological findings in organs of *Clarias gariepinus* fingerlings fed Auto-detoxified Jatropha kernel cake/ bovine blood (ADJKC/bb) mixture based diets for 8 weeks.

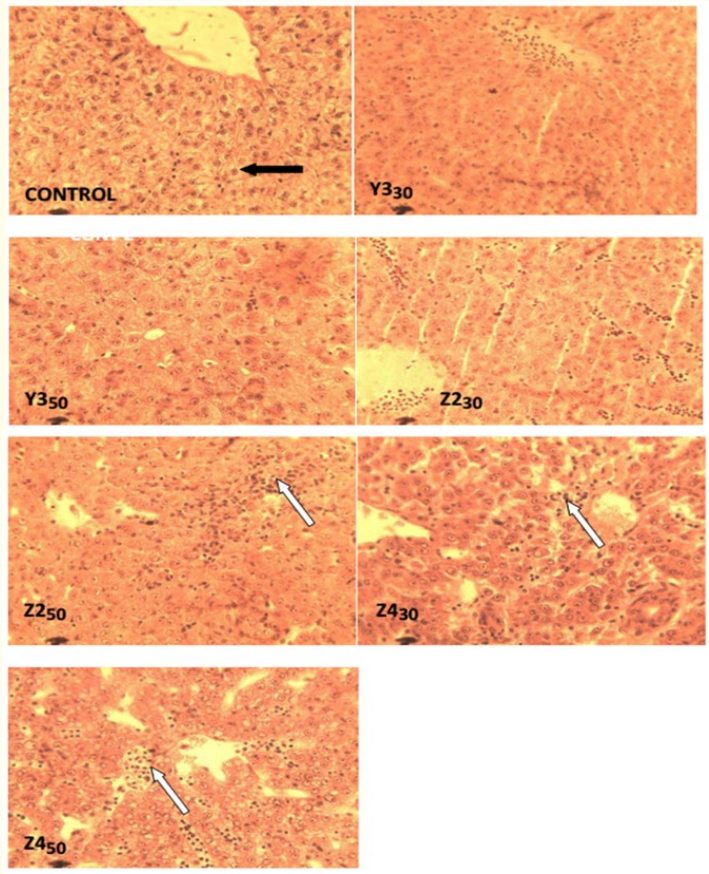
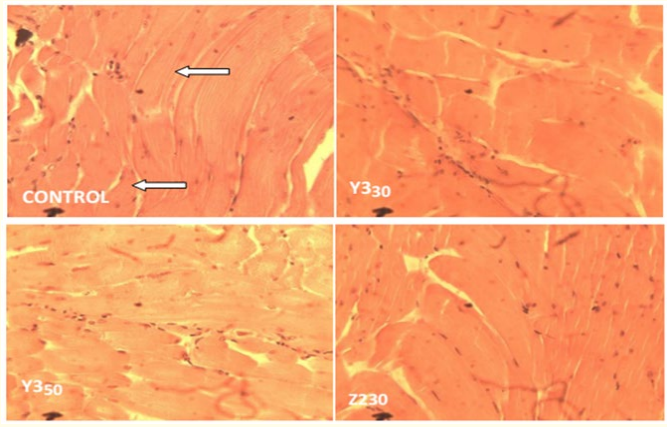


Figure 1: A photomicrograph of liver sections from *Claris gariepinus* fingerings fed ADMJKC/bb mixture based diets for 8 weeks: showing the control with normal histology and glycogen rich cytoplasm (black arrows), $Z2_{50}$, $Z4_{30}$ and $Z4_{50}$ showing focal areas of hepatocyte necrosis and mononuclear cell infiltrration (white arrows), while $Y3_{30}$, $Y3_{50}$ and $Z2_{30}$ are apparently normal. Stain = haematoxylin and eosin; Magnification = 400X.



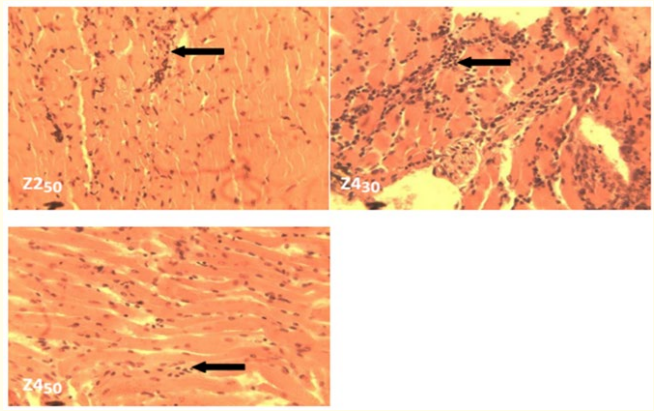


Figure 2: Photomicrograph of muscle sections from *Claris gariepinus* fingerings fed ADMJKC/bb mixture based diets for 8 weeks: showing the control with normal histology of the myofibres (white arrows), $Y3_{30}$, $Y3_{50}$ and $Z2_{30}$ are apparently normal, while $Z2_{50}$, $Z4_{30}$ and $Z4_{50}$ show mild to moderate muscle degeneration and necrosis with mononuclear cell infiltration between the myofibres (black arrows). Stain = haematoxylin and eosin; Magnification = 400X.

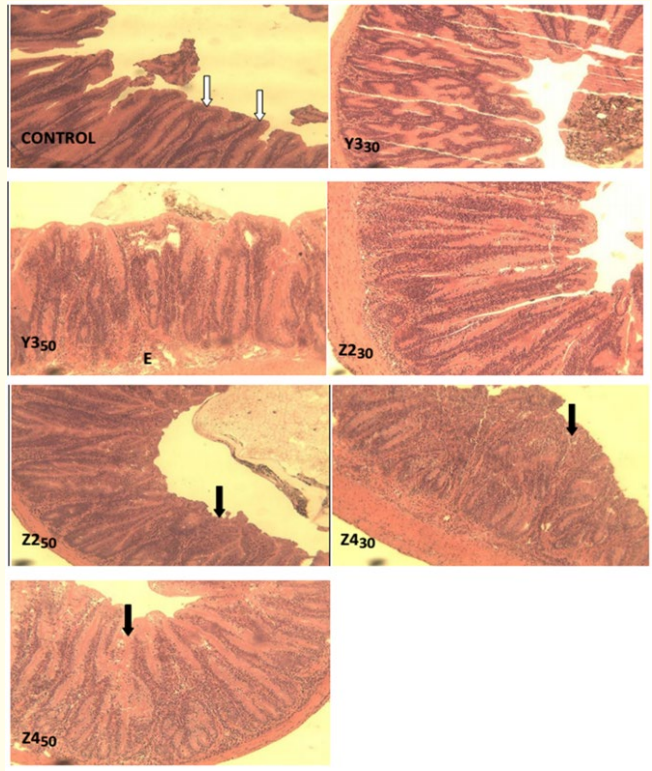


Figure 3: Photomicrograph of intestinal sections from *Claris gariepinus* fingerings fed ADMJKC/bb mixture based diets for 8 weeks: showing the control with normal histology, (white arrows). Groups $Z2_{50}$, $Z4_{30}$ and $Z4_{50}$ show necrosis of enterocytes and sloughing / denudation of the tips of the villus which led to reduction in their sizes (black arrows). Note the mild oedema of the submucosa and lamina propria in $Y3_{30}$ (E). Stain = haematoxylin and eosin; Magnification = 400X.

Discussion

The control was significantly (P<0.05) higher in Hb, PCV and MCV than the ADMJKC/bb diets. However, among the ADMJKC/bb diets, significant (P<0.05) differences between the 30% and the 50% inclusion levels, were recorded in PCV and WBC for all ingredients. Haematological parameters such as PCV and WBC are indicators of toxicity, in aquatic animals (Sancho et al., 2000). Haematocrit (PCV) is the quickest and easiest way to determine the number of red blood cells in the whole blood. Low PCV indicates anaemia (Frank and Solomon, 2017). This may imply that the 50% ADMJKC/bb diets induced a tendency for fishes to be anaemic. Chivandi et al. (2006) observed anaemic tendencies in pigs fed Jatropha. Also, Omitoyin (2006) observed a similar anaemic condition in *C. gariepinus* fed poultry litter; and inferred that there was protein inadequacy from poultry litter which might have inhibited erythrocyte production or increase rate of destruction. In this study, the main difference in protein quality between the 30% and the 50% ADMJKC/bb diets is the quantity of JKC. The test ingredients may therefore influence haematological parameters via nutrients as well as bound anti-nutrients.

The control and the 30% levels of ADMJKC/bb diets were similar (P>0.05) in ALP, but different (P<0.05) in ALT. Also, there was a wide disparity in the numeric values between levels in ALP, although all the values in ALP and ALT were within the normal range given for *C. gariepinus* (Myburgh et al., 2008). In mammals, ALT and ALP tend to be useful markers of hepatocellular pathology and biliary pathology respectively (Latimer et al., 2003). Alanine aminotransferase levels are also known to vary according to age, sex (Dufour et al., 2000), strenuous exercise (Dufour, 1998) and diet (Giannini et al., 2005). However, the actual numeric value of plasma enzyme activity is poorly correlated with the degree of tissue damage (Myburgh et al., 2008). Therefore, the variation in ALP numerical values between levels of ADMJKC/bb diets as well as the similarity in the trend with other haematological data is too striking to be ignored. Surely, the nutrients and bound anti-nutrients in ADMJKC/bb ingredients based diets are implicated in hepatobilliary pathology.

The control was similar (P>0.05) to the 30% ADMJKC/bb diets in RIL and ISI, while $Z2_{50}$ and $Z4_{50}$ were significantly (P<0.05) higher than the control in both parameters. This response of the fish may be associated with increasing level of plant proteins in the diets. The $Z2_{50}$ and $Z4_{50}$ diets contained three parts of JKC to one part of bovine blood. This high quantity of JKC could also present a higher quantity of un-detoxified lectins to the intestines. Letcins are known to cause morphological changes in the intestine and therefore reduce the absorption of nutrients. During intestinal transit, about 60% of the lectins bind to the brush border membranes of the intestines. Here, it disrupts the membrane and causes atrophy of the microvi of microvilli. This reduces viability of epithelial cells. Consequently, interaction of epithelial cells with lectins causes an increase in the weight of the small intestine (Gogoi et al. 2015). The ISI is a weight response parameter and its surge in $Z2_{50}$ and $Z4_{50}$ diets may therefore, confirm the presence of lectins . Aregheore et al. (2003) demonstrated that lectin activity was significantly decreased by defatting, followed by heating of Jatropha seeds. This may explain the lower ISI for the Y3 ADMJKC/bb diets because the Y3 ADMJKC/bb ingredients were pre-processed by heating unlike their Z2 and Z4 counterparts.

The HSI increased with the 50% level of fishmeal replacement. Though this was not significant (P>0.05), it is an indication of a decrease in the energy reserves of the fish at higher levels of ADMJKC/bb diets (Sogbesan et al., 2017). In a poor environment, fish usually has smaller liver with less energy reserved (Junaid et al., 2006). This decrease in energy reserves could be resulting from extra effort to detoxify bound anti-nutrients.

Further evidence of use of energy reserves is the significant decrease in Spleen Somatic Index (SSI), with the 50% ADMJKC/bb diets. Spleen is a key storage organ for blood cells. Rohlenova et al. (2011) stated that production of antibodies showing immune reactivity in response to pathogens and foreign particles in blood stream is yet another vital function of spleen. The spleen is known to typically increase in proportion to body weight of an animal (Weatherley and Gill, 1983). However, during periods of acute stress it contracts (Pearson and Stevens, 1991). Stress could result from need to detoxify some anti-nutrients which subjects the fish to additional pathological challenges. The spleen contracts (reduction in spleen somatic index) to release more leucocytes in the general blood circulation to overcome this stress. This action uses up a lot of the fish energy. This may explain why the spleen is one of the main organs affected by PE toxicity (Devappa, 2012), and also probably accounts for the significantly (P<0.05) higher WBC count, with 50% ADMJKC/bb diets.

The liver of the control group showed moderate signs of increased glycogen reserves. Conversely, the ADMJKC/bb diets showed mild signs of decreased glycogen. This is an indication of malnutrition among the ADMJKC/bb fed fishes. Malnutrition observed from the liver, results from reduced intake, absorption, processing and storage of nutrients (Saunders et al., 2010). Reduced glycogen storage also increases amino acid needs for gluconeogenesis while on-going inflammation alters the pattern of amino acid requirements, precipitating specific amino acid shortages (Reeds et al. 1994). Phorbol esters which are the main toxic components of JKC are known to exert their harmful effect by mimicking diacylglycerol (DAG) in the activation of protein kinase C (PKC) (Zhang et al., 1995). However, unlike DAG whose effect is transient, the impact of PEs on PKC is much more prolonged (Griner and Kazanietz, 2007), and this leads to a cascade of biological activities that are more energy demanding. The ADMJKC/bb diets probably contain some residual PEs and other anti-nutrients, whose detoxification is responsible for the decreased liver glycogen.

The level of liver necrosis changed from mild to moderate, and from moderate to severe from $Z2_{30}$, through $Z2_{50}$ and $Z4_{50}$, while it remained mild for $Y3_{30}$ and $Y3_{50}$ but was absent in the control. Additionally, mononuclear cell infiltrates were absent in the control and Y3 ADMJKC/bb diets, but changed from absent to mild and moderate to moderate with increasing levels of Z2 and Z4 ADMJKC/bb diets respectively. This is evidence of response to both proportion of JKC among the ADMJKC/bb and their methods of processing. The Y3 ingredients were composed of two parts JKC and one part bovine blood, while the Z2 and Z4, contained three parts JKC to one part bovine blood. Furthermore, Y3 was processed by heating, spread dried, and remoistened daily to 66% DM, while Z4 was processed without heating, but was spread dried, and remoistened daily to 66% DM. On the other hand, Z2 was spread dried without heating and remoistening. It is possible therefore, that the three ingredients presented different metabolic challenges to the liver with particular reference to detoxification and transamination. This may perhaps explain why the control and lower levels of ADMJKC/bb ingredients based diets were statistically similar ($P>0.05$) in ALP but they were significantly ($P<0.05$) different in ALT.

Hepatic necrosis is death of hepatic parenchyma. It may be single cell (necrobiosis) or multicell (piecemeal, focal, periacinar, midzonal, periportal or paracentral) in location. Focal necrosis is a common feature in catfish (Olojo et al., 2005). Liver necrosis occurs when hepatotoxic substances or metabolites react with macromolecules in hepatocytes (Brodie et al., 1971). It is one of the pathological observations when *C. gariepinus* is exposed to toxins such as lead (Olojo et al., 2005). The direct toxic effect of phorbol esters probably led to degeneration and necrosis of hepatocytes in this present study. It was similar to the observation in rats by Aregheore et al. (2003). Thus the liver necrosis is further proof that ADMJKC/bb diets contained some phorbol esters. The necrosis resulted from the excessive work required by the fish to handle the cascade of biological reactions ensuing from the effect of phorbol esters mimicking DAG in the liver cells in combination with the inability of the fish to regenerate new liver cells at a rate commensurate with the destruction.

In the muscle, necrosis, degeneration and mononuclear cells infiltration were all absent in the control and mild in the Y3 ADMJKC/bb diets, irrespective of levels of fishmeal replacement. However, they moved from mild to moderate for the Z2 but remained severe for the Z4 diets. Khalil et al. (2016) made a similar observation, for fantail goldfish (*Carassius auratus L.*) that were undergoing starvation.

The inflammatory cell infiltration and vilus atrophy increased in severity with increasing ADMJKC/bb ingredients in the intestines. However, the intestinal pathology of the fishes fed the Z4 diets, was more severe than the Z2 and Y3 diets. The pathology observed possibly resulted from the effects of phorbol esters and other phytotoxins, on the cellular membrane receptors, with modification of their activities, which led to release of the different inflammatory mediators, including histamine, resulting in vascular disturbance, inducing oedema (Goel et al., 2007).

The main toxic components of Jatropha, is phorbol esters. Histopathological studies of the organs have shown a dosage response, to administration to purified phorbol esters. The phorbol esters are amphiphilic molecules and tend to bind to phospholipid membrane receptors, which usually are their primary target (Azzaz et al., 2011). Li et al. (2010) concluded that all the feeding studies on *J. curcas* showed severe clinical and pathological symptoms. Among the critical symptoms observed was transient loss of body mass and mild to severe macroscopic/ microscopic changes in the kidney, lungs, heart, liver, and spleen in a dose dependent manner. The symptoms and toxicity of *J. curcas* depend on extract, dose, mode of administration, and sensitivity of test animals (Li et al., 2010).

Conclusion

The haematological, histopathological, and internal organ observation in this study, lead to the conclusion that the Y3 and Z2 ADMJKC/bb ingredients were better detoxified than the Z4 ingredient. However, Y3 and Z2 should not replace more than 30% of fishmeal in diets for *Clarias gariepinus* fingerings. There is need therefore to continue development of the Y3 and Z2 ingredients for *C. gariepinus* and other farm animals. The test ingredients may be influencing hematological, histopathoogical and internal organs of *C. gariepinus* through their nutrient as well as bound anti nutrient content.

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