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Growth Performance and Histopathology of *Clarias Gariepinus* Fed Toasted *Canavalia Ensiformis* Diet

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ABSTRACT: *Fish farms output and profits have been facing a greater challenge with a hike in the price of feed ingredients. This study was designed to evaluate the growth performance and histopathology of clarias gariepinus fingerlings fed Toasted Canavalia ensiformis seed meal diets.Toasted Canavalia ensiformis meals were used to formulate five different 40.0% isoproteic diets each at 0%, 25%, 50%, 75% and 100% and coded TCe, which were fed at 5% body weight to fingerlings of Clarias gariepinus fingerlings. One hundred and fifty Clarias gariepinus (0.96g±0.03 and 3.93cm±0.28) were randomly assigned to five treatments of 10 fingerlings, replicated three times in a completely randomised design (CRD) in 35L plastic circular bowls through a semi-flow-through system for 84days. Weights and lengths were measured biweekly and used to calculate the growth and nutrient utilization parameters. Histopathology of the fish organs were evaluated. Data collected were analysed using a one-way analysis of variance. Results from the statistical analysis revealed significant differences ($p<0.05$) in the specific growth rate as the inclusion levels of the tested meals increased. Histopathological changes in the liver cells (hepatocytes) such as mild and severe diffuse vacuolations of the hepatocytes, vacuolar degeneration. Tubule disruption and necrosis were observed in the kidney of fish, blood congestion, cellular hypertrophy, loss of epithelial cell and coagulation necrosis of the lamellae was observed in the gills.This study, therefore, suggested that Clarias gariepinus fingerlings can feed on up to 25% inclusion level Toasted Canavalia ensiformis diet without adverse effects on the growth performance.*

KEYWORDS: Growth performance, Histopathology, Toasted, *Canavalia ensiformis*, *Clarias gariepinus*

INTRODUCTION

The need for plant protein in fish feed is essential because it is more available and affordable. The search for protein-rich plant source is an ongoing process, leaf meal inclusion in aquaculture feed also is fast gaining global attention over the years due to its availability, protein and mineral/vitamin contents and economic feasibility (Ali and Kumar 2000). Several studies have been conducted on the use of terrestrial and aquatic leaf meals as Dietary protein sources in fish feed. The extent of plant protein utilization is also influenced by its availability, ease of processing and nutritive value, nutrient bioavailability and its acceptability by fish

(Screnson *et al*. 2009). Since the primary objective of fish nutrition is reduction of protein cost in fish feeds which has been adjudged to cost about 75% of the cost of feed (Adejinmi *et al*. 2001; Sogbesan 2014).

Legumes have been recognized to be the second most valuable plant source for human, animal nutrition and the third largest family among flowering plants, consisting of approximately 650 genera and 20000 species (Vietmeyer, 1986; Doyle, 1994). Kalidass and Mohan 2012, reported that legume seeds are important sources of nutrients and can serve as high quality dietary protein to meet nutrient requirements of fishes (Perumal *et al*. 2001; Escudero *et al*. 2006. These seeds have an average of twice protein content as in cereals and the nutritive values of the proteins in legumes are usually higher (Vijayakumari *et al*. 1997). The wild legumes, which have tremendous potential for commercial exploitation but remain ignored, form a good scope in this context (Bhag, 1992). Ali and Kumar 2000 reported that common proteinaceous edible legumes (soybean, cowpea, and others) are available in the market, and in most cases, production rates are compared with consumption (as food and feed) has remained unmet, and an ever-increasing demand has been witnessed by food and feed industries. Also, switching by most of the world's population to a protein-rich vegetarian-based diet from animal-based protein has created unwarranted scarcity to plant protein resources. In this regard, legumes have been highlighted as an effective substitute to animal protein (Famurewa and Raji 2005). Considering the above, it becomes imperative for nutritionists to search for cheap, reliable, and safe plant-based resources to accomplish the demand for protein-rich feed. Researchers throughout the world are concentrating their efforts on tapping natural wild and underutilized legumes, which have remained either unexplored or underutilized or localized in a particular region for alleviating hunger and to overcome malnutrition (Coulter *et al*. 1988; Chel-Guerro *et al*. 2002; Arinathan *et al*. 2003. Exploring under-utilized legumes could be of high significance for food security, meeting nutritional requirements, agricultural development and thus can effectively contribute to the overall improvement of a nation's economy. Many of the known wild legumes (such as *Mucuna* spp., *Canavalia* spp., *Sesbania* spp., *Jatropha* spp.) possess adequate amounts of protein, essential amino acids, polyunsaturated fatty acids (PUFAs), essential minerals and vitamins comparable to other common legumes, along with the presence of beneficial bioactive compounds. Apart from this, these plants are also adaptable to adverse environmental conditions and can thrive under extreme stress conditions (Amubode and Fetuga 1983; Sotelo *et al*. 1999; Bhat *et al*.2008).

Canavalia ensiformis is an annual or weak perennial legume with climbing or bushy growth forms. It is woody with a long tap root. The 8 in (20 cm) long and 4 in (10 cm) wide leaves have three egg-shaped leaflets, are wedge-shaped at the base, and taper towards the tip. The 1 in (2.5 cm) long flowers are rose-colored, purplish, or white with a red base. It has a 12 in (30 cm) long, 1.5 in (3.8 cm) wide, sword-shaped seed pod. Seeds are white, red, brown and smooth with a brown seed scar that is about one-third the length of the seed. Its roots have nodules which fix nitrogen (Fagbenro *et al*. 2004; Tiamiyu *et al*. 2016). The genus *Canavalia* comprising of 48 species of these underutilized legumes. They are widely distributed and indigenous to the tropics (Fagbenro *et al*. 2004), rarely eating by man (Okonkwo and Udedibie, 1991) and their nutritional potential has been well studied in monogastrics and poultry industry (Udedibie, 1990; Udedibie and Nkwocha 1990). Nutritional trial in fish includes the works of

Martinez-Palacios *et al*. 1998, Akinbiyi, 1992; Abdo de la Parra *et al*. 1998; Osuigwe *et al*. 2002 and Fagbenro *et al*. 2004. Evaluation of histological structure of digestive organs in fish fed new dietary ingredients generates valuable information about their digestive capacity and the potential health effects of such diets. Diaz *et al.* 2006. Uwachukwu *et al.* (2003) reported that diets containing raw beans caused extensive periportal necrosis with some mononuclear cell infiltration in the livers of broilers while the centrilobular areas showed vacuolation and degeneration of hepatocytes. Olasunkanmi, 2015 also observed that *C. gariepinus* fed processed velvet beans showed both mild diffuse and marked widespread vacuolation of the hepatocytes. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no regenerative ability (Nayak *et al*. 1996) and the excessive vacuolation of the hepatocytes would result in abnormal functioning of the liver cells, for example, immobilization of fat, which could consequently result in fatty infiltration of the hepatic parenchyma (Adeyemo, 2005).

MATERIALS AND METHODS

Study area: The feeding trial was conducted at the Research Farm of the Department of Fisheries, Moddibo Adama University (MAU, Yola). Adamawa State is located on latitude 9.14^0N, longitude 12.38^0E and an altitude of 185.9m. Girei is located on latitude 9.22^0N, longitude 12.33^0E and altitude of 245m. It has an average annual rain fall of about 759mm with maximum temperature of 39.7^0C. The rainy season run from May through October, while the dry season commences November and ends in April. The driest months of the year are January and February when the relative humidity drops to 13% (CGIDD, 2014).

Seed Collection and Identification: *Canavalia ensiformis* fruits were collected from Girei and surroundings in Adamawa State. They were identified using a field handbook by Arbonnier (2004) by plant Taxonomist in Forestry and Wildlife Department of Modibbo Adama University, Yola (MAU).

Preparation of the Legume Seeds and Processing: The seeds were dehulled, clean of dirts by hand picking and winnowed. The seed size was reduced with pestle and mortar, and subjected to various processing methods according to Doss *et al*. 2011 and Antyev, 2018 methods:

1. Raw seeds were milled and tag raw seed meal (RSM)
2. Raw seeds were soaked in water to the ratio of 1:3 for 72hours, oven dried at 50^oC to constant weight then milled and tag soaked seed meal (SSM)
3. Raw seeds were boiled for 30minutes, oven dried at 50^oC to constant weight then milled and tag boiled seed meal (BSM)
4. Raw seeds were toasted at 70^oC using electric hot plate until seeds turn brown in colour then milled and tag Toasted seed meal (TSM)
5. Raw seeds were moistened with water, kept in a container with cover to fermented for 72 hours under laboratory condition, oven dried at 50^oC then milled and tag fermented seed meal (FSM)

Analysis of Nutrient Compositions and Anti- Nutritional Factors: The seed meals were analyzed for nutrient compositions and anti-nutritional factors according to AOAC, 2012 methods.

Feed formulation: Basal diet of 40% crude protein were formulated from the commercial ingredients. Toasted *Canavalia ensiformis* were added to the experimental diets to replace *Glycine max* meal as plant protein source at inclusion levels of 0% (control), 25%, 50%, 75% and 100% as shown in Table 1.

Table 1: Percentage Compositions of Ingredients with Toasted *Canavalia ensiformis* Meal

	Inclusion levels				
Ingredients (g/100g)	Control (0%)	TCe (25%)	TCe (50%)	TCe (75%)	TCe (100%)
Fishmeal (68%)	32.00	32.00	32.00	32.00	32.00
Glycine max meal (46%)	33.00	24.74	16.50	8.25	0.0
TCe Meal (35%)	0.0	10.93	21.72	32.53	43.37
Yellow maize (10%)	30.00	27.33	24.78	22.22	19.63
*Vitamin/mineral premix	1.0	1.0	1.0	1.0	1.0
Lysine	0.5	0.5	0.5	0.5	0.5
Methionine	0.5	0.5	0.5	0.5	0.5
Palm oil	1.0	1.0	1.0	1.0	1.0
Cassava starch	1.0	1.0	1.0	1.0	1.0
Dicalcium phosphate	0.5	0.5	0.5	0.5	0.5
Common salt	0.5	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0	100.0
Calculated Crude protein	39.94	39.69	39.43	39.16	38.89
**Calculated Gross Energy (KJ/100g)	1868.60	1818.80	1769.70	1720.70	1672.20
GE:CP	46.78	45.84	44.91	43.94	42.98

TCeM – Toasted *Canavalia ensiformis* meal
GE: CP= gross energy: crude protein
**Calculated Gross Energy (KJ/100g) = Protein x 23.6KJ/100g + Lipid x 39.5KJ/100g + NFE x 17.2KJ/100g (Blaxter, 1989)

*Vitamin-Mineral premix provides per kg the following: 12.000.000 IU Vitamin A; 2.000.000 IU Vitamin D3; 10g Vitamin E; 2g Vitamin K3; 1g Vitamin B1; 5g Vitamin B2; 1.5 g Vitamin B6; 10g Vitamin B12; 30g Nicotinic acid; 10g Pantothenic acid; 1g Folic acid; 50g Biotin; 250g Choline chloride 50%; 30g Iron; 10g copper; 50g Zinc; 60g Manganese; 1g Iodine; 0.1g Selenium and Cobalt 0.1g.

Growth Parameters: The initial weight and length of fish in each treatment were taken at two weeks' interval. The feed rations fed were adjusted based on the weight. The weight and length recorded were used to determine the growth performance of the fishes and feed supplied were also used to determine the nutrient utilization parameters following the methods of Aderolu *et al.* 2010.

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Histopathological Examination

***Collection of Organs*:** The same fish samples that were used for the haematological examination were dissected to remove the gills, liver, kidney and heart. The organs were removed, weighed separately and recorded for somatic indices.

Organ Somatic Indices: The ratio of the weight of the liver, kidney and heart in relation to the body of fish were measured separately using the following: Gill somatic index (GSI), kidney somatic index (KSI), Liver somatic index (LSI) and Hepatosomatic index (HSI) as described by Sogbesan, 2014:

$$\text{Organosomatic index (\%)} = \frac{\text{Organ Weight (g)}}{\text{Body Weight (g)}} \times 100$$

Procedure for Histopathological Examination: Histopathological Examination was carried out to investigate possible abnormalities in the Gills, Livers, kidneys and hearts of fishes fed the different experimental diets. The organs were fixed in 10% neutral buffer formalin for 24 hours and slides preparation were done at Department of Biological Sciences, Ahmadu Bello University, Zaria. Each organs was dehydrated in periodic acid Schiffs reagent (PAS) following the method of Hughes and Perry, (1976) cleaned, impregnated with wax and sectioned with a microtome (5 µm thickness). Sections was then stained with Harris haemotoxylin-eosin (H$E) (Bancroft and Cook, 1994) for histopathological examination of the liver, kidney and heart. The cytological qualities of these organs were assessed using Freeman and Bracegirdile, 1981. Photomicrography of the sections was taken with a digital camera, attached to Olympus BH2 binocular light microscope at different magnification.Data collected were subjected to oneway analysis of variance ANOVA. Significant differences between treatment means were compared using least significant difference at 5% probability level using IBM SPSS statistics 19.

RESULTS

Table 2: Proximate Compositions of Toasted *Canavalia ensiformis* Diets on Dry Matter Basis

Nutrients	TCe 0%	TCe 25%	TCe 50%	TCe 75%	TCe 100%
Protein	40.36	40.20	40.00	39.02	39.00
Lipid	7.01	6.76	6.83	7.04	6.97
Fibre	5.43	5.68	5.71	5.76	5.78
Ash	6.52	6.53	6.60	6.75	7.02
NFE	32.18	32.33	32.16	32.81	32.74
Dry Matter	91.5	91.5	91.3	91.38	91.51
Calculated analysis					
Gross Energy (Kcal/g)	417.71	415.17	414.01	413.19	412.16
Digestible Energy (Kcal/g)	273.81	271.50	271.86	270.23	269.44
Metabolizable Energy (Kcal/g)	3202.52	3182.67	3174.91	3178.73	3169.84

TCe - Toasted *Canavalia ensiformis*

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Table 3: Growth Indices and Survival Rate of *Clarias gariepinus* Fingerlings Fed Toasted *Canavalia ensiformis* Diets for Three Months

Parameters	Control 0%	Tce 25%	Tce 50%	Tce 75%	Tce 100%	SEM
Total initial weight (g)	9.53±0.50	9.6±0.52	9.53±0.50	9.66±0.57	9.6±0.52	0.30^{ns}
Total final weight (g)	$61.6±6.03^{a}$	$31.73±1.22^{b}$	$27.2±2.4^{b}$	$14.33±0.76^{c}$	$9.76±1.56^{c}$	1.76^{***}
Mean initial weight (g/fish)	0.96±0.05	0.96±0.05	0.96±0.05	1.0±0.10	0.96±0.05	0.03^{ns}
Mean final weight (g/fish)	$7.70±0.75^{a}$	$3.96±0.15^{b}$	$3.40±0.30^{bc}$	$2.86±0.15^{cd}$	$2.66±0.05^{d}$	0.22^{***}
Mean weight gain (g/fish)	$6.74±0.70^{a}$	$3.0±0.10^{b}$	$2.44±0.25^{bc}$	$1.86±0.05^{cd}$	$1.7±0.00^{d}$	0.21^{***}
Mean Biweekly weight gain (g/fish/week)	$0.56±0.70^{a}$	$0.25±0.10^{b}$	$0.20±0.25^{bc}$	$0.15±0.05^{cd}$	$0.14±0.00^{d}$	0.02^{***}
Mean initial length (cm/fish)	3.93±0.50	3.96±0.47	3.86±0.55	3.86±0.40	3.93±0.51	0.28^{ns}
Mean final length (cm/fish)	$7.9±1.25^{a}$	$6.26±0.51^{b}$	$6.43±0.70^{b}$	$6.26±0.47^{b}$	$6.26±0.05^{b}$	0.41^{ns}
Relative growth rate (%/fish)	$769.07±0.70^{a}$	$319.58±0.10^{b}$	$274.22±0.25^{c}$	$200±0.05^{d}$	$195.87±0.00^{d}$	18.82^{***}
Specific growth rate (%/day)	$1.1±0.70^{a}$	$0.71±0.10^{b}$	$0.7±0.25^{b}$	$0.55±0.05^{c}$	$0.52±0.00^{c}$	0.03^{***}
K1	1.59	1.56	1.68	1.73	1.59	0.31^{ns}
K2	1.7	1.65	1.36	1.22	1.16	0.13^{ns}
Survival (%)	80^{a}	80^{a}	80^{a}	30^{b}	10^{c}	1.49^{***}

Mean ± Std on the same row with different superscripts are significantly different (P<0.001)
***, ^{ns} = not significant (p>0.05), SEM- standard error of mean,
TCe- Toasted *Canavalia ensiformis*

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Table 4: Feed Intake and Nutrient Utilization Indices of *Clarias gariepinus* Fingerlings fed Toasted *Canavalia ensiformis* (TCe) Diets for Three months

Parameters	Control 0%	Tce 25%	Tce 50%	Tce 75%	Tce 100%	SEM
Total feed intake (g)	1187.76^{a}	671.16^{b}	577.92^{c}	412.44^{d}	340.2^{e}	0.28***
Mean feed intake (g/fish)	14.14±0.17^{a}	7.99±0.13^{b}	6.88±0.12^{c}	4.91±0.19^{d}	4.05±0.14^{e}	0.01***
Biweekly feed intake (g/week)	197.96^{a}	111.86^{b}	96.32^{c}	68.74^{d}	56.7^{e}	0.05***
Voluntary feed intake (g/fish)	1.94±0.21^{a}	1.93±0.65^{a}	1.87±0.34^{a}	1.51±0.76^{b}	1.33±1.4^{b}	0.06**
Feed acceptability index (%)	0.229±0.02^{a}	0.147±0.01^{b}	0.141±0.01^{c}	0.092±0.01^{d}	0.085±0.01^{e}	0.01***
Feed conversion ratio	2.07±0.01^{c}	2.66±1.3^{ab}	2.84±0.48^{a}	2.65±3.8^{ab}	2.38±0.00^{bc}	0.14**
Protein intake (g/100g diet/fish)	570.6±0.01^{a}	321.1±0.01^{b}	275.2±0.01^{c}	191.5±0.01^{d}	157.9±0.01^{e}	0.18***
Protein efficiency rate	0.16^{a}	0.066^{b}	0.056^{bc}	0.046^{c}	0.040^{c}	0.01***

Mean ± Std on the same row with different superscripts are significantly different (P<0.01) ^{xx}, (P<0.001) ^{xxx}, SEM- standard error of mean,
TCe- Toasted *Canavalia ensiformis*

Liver: Photomicrograph of a section of the liver of *C. gariepinus* fed 0% TCe-based diet (control diet) showed Hepatocytes. Fish fed 25% TCe-based diet showed blood coagulation. Fish fed 50% diet showed Coagulation necrosis of the hepatic cell and fibrosis (Plate I).

Heart: Photomicrograph of a section of the Heart of *C. gariepinus* fed 0% (control diet) and 25% TCe-based diet showed Artery and Adrenal cell. Fish fed 50% TCe-based diet showed shrinkage of Artery (Plate II).

***Kidney*:** Photomicrograph of a section of the Kidney of *C. gariepinus* fed 0% TCe-based diet (control diet) showed Glomerulus, Tubules and Hematopoietic tissue. Fish fed 25% TCe-based diet showed increase in renal tubule. Fish fed 50% diet showed shrinkage of renal corpuscle (Plate III).

Gills: Photomicrograph of a section of the gills of *C. gariepinus* fed 0% TCe-based diet (control diet) showed primary lamella, primary epithelium and secondary lamellae. Fish fed 25% TCe-based diet showed blood congestion and cellular hypertrophy. Fish fed 50% diet showed loss of epithelial cell and coagulation necrosis of the lamellae (Plate IV).

Table 5: Organosomatic Indices of *Clarias gariepinus* Fed TCe Diets

Indices	Control 0%	TCe 25%	TCe 50%	SEM
Liver somatic index (%)	60.53±1.05[a]	44.3±1.10[b]	42.4±1.11[b]	0.63[***]
Heart somatic index (%)	40.6±1.05	40.0±1.00	40.0±1.00	0.58[ns]
Kidney somatic index (%)	8.53±1.00	7.50±1.10	7.30±0.95	0.58[ns]
Gill somatic index (%)	89.0±1.00[a]	85.5±0.85[b]	84.4±1.05[b]	0.56[***]

Mean ± Std on the same row with different superscripts are significantly different (P<0.001) [***], SEM- standard error of mean, [ns] = not significant (p>0.05)

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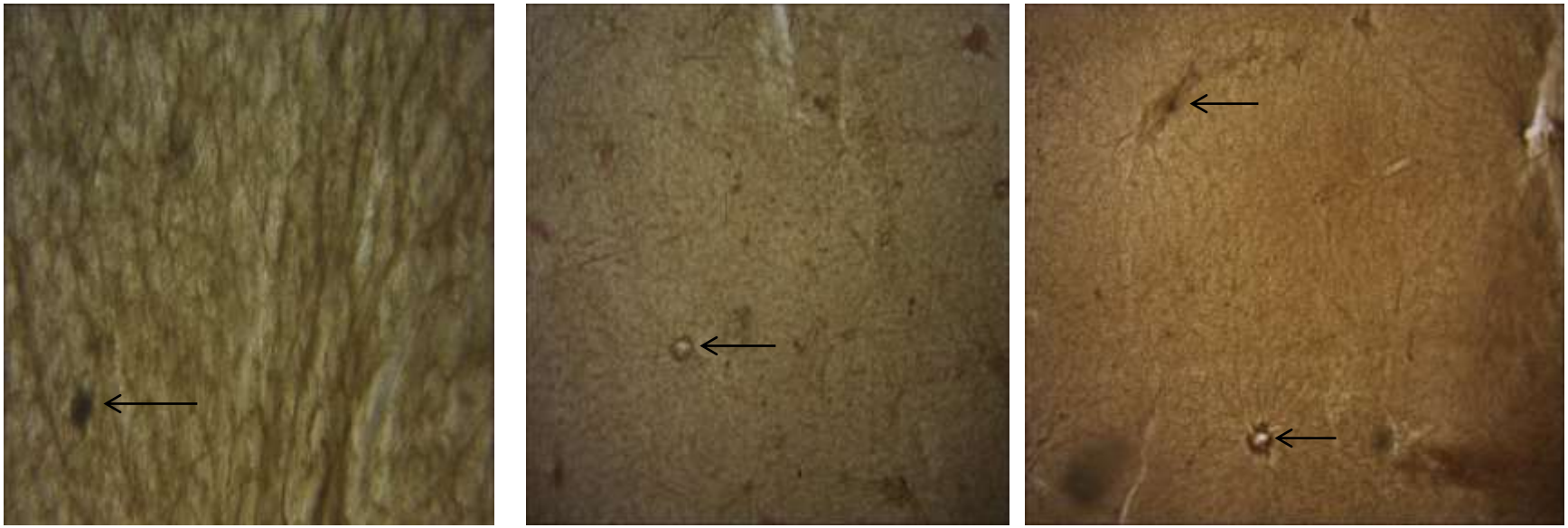
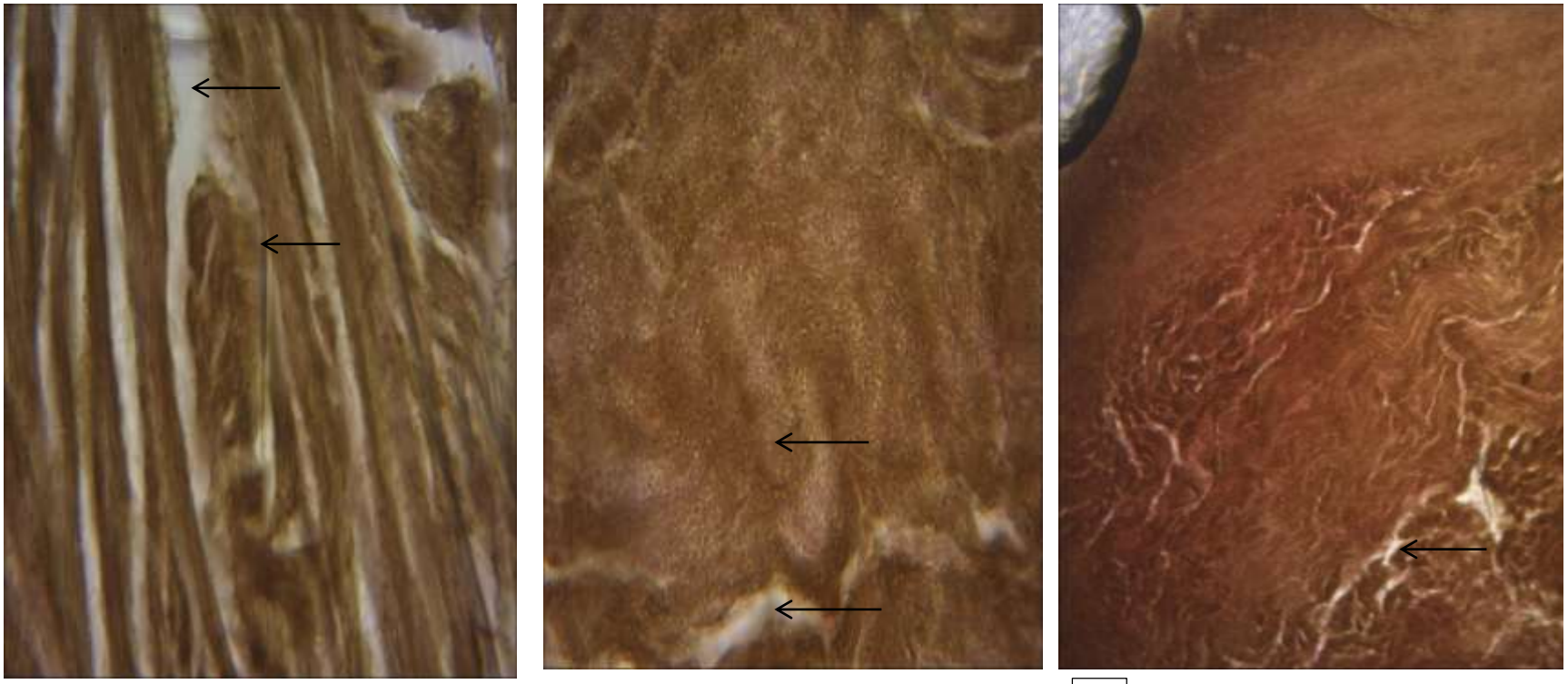
TCe- Toasted *Canavalia ensiformis*



A B **C**

Keys:

Plate I: Liver Histology of *Clarias gariepinus* Arror showing: (A) Control Hepatocytes. (B) *Clarias gariepinus* Fed TCe 25% Diet Blood Coagulation. (C) TCe 50% Coagulation Necrosis of the Hepatic Cell and Fibrosis. H&E X400.



A **B** **C**

Keys:

Plate II: Heart Histology of *Clarias gariepinus* Arror showing: (A) Control Structural Arrangement of Artery and Adrenal cell. (B) *Clarias gariepinus* Fed TCe 25% Diet Artery and Adrenal cell. (C) TCe 50% showing Shrinkage of Artery. H&E X400.

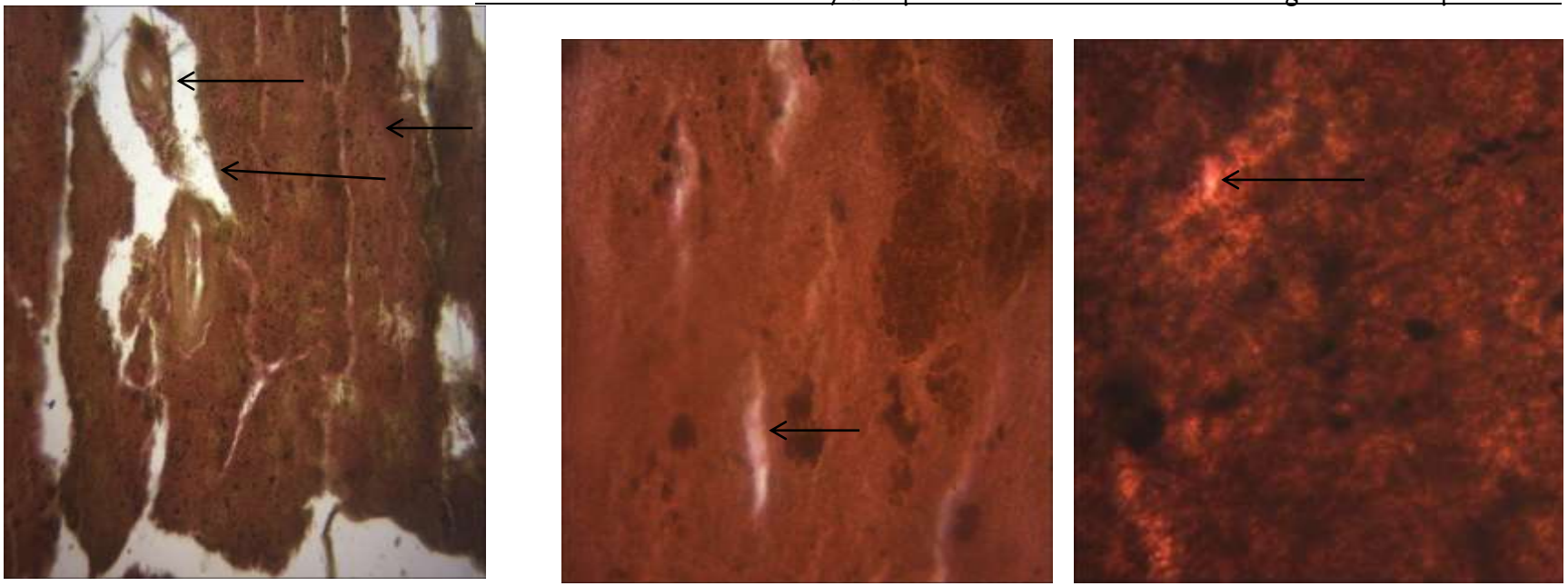
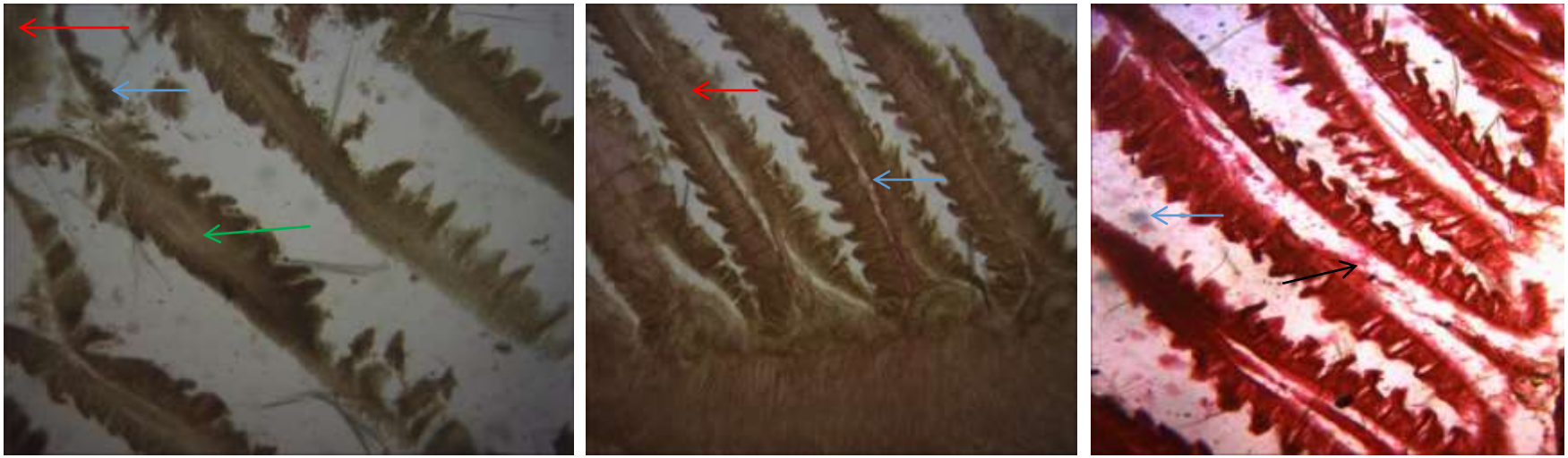


Plate I: Kidney Histology of *Clarias gariepinus* After showing: (A) Control Glomerulus, Tubules and Hematopoietic Tissue. (B) *Clarias gariepinus* Fed TCe 25% Diet Increase in Renal Tubule. (C) TCe 50% showing Shrinkage of Renal Corpuscle. H &E X400.



A B C

Keys:

Plate IX: Gill Histology of *Clarias gariepinus* Arror showing: (A) Control Primary Lamella (red), Primary Epithelium (Blue) and Secondary Lamella (green). (B) *Clarias gariepinus* Fed TCe 25% Diet with Blood Congestion (Blue) and Cellular Hypertrophy (red). (C) TCe 50% showing Loss of Epithelial Cell (Blue) and Coagulation Necrosis of the Lamellae (Black). H &E X400.

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DISCUSSION

Growth Performance and Nutrients Utilization of Clarias gariepinus Fed Toasted Canavalia ensiformis (TCe) Diets

Growth performance and nutrient utilization as observed revealed significant reduction in performance as *C. ensiformis* meal inclusion increases. It was observed that fish fed toasted *C. ensiformis* diet performed less than the control which agree with the report of Olukunle *et al*. 2015, Tiamiyu *et al*. 2016. Fagbenro *et al*. 2004 had earlier opined that biological indicators such as growth performance, survival, feed utilization efficiency, nutrient availability, gross or sub-clinical abnormal signs are basic means employed in determining the efficacy and adequacy of diets fed. Hence, it may be concluded that the presence of antinutrients and reduction of amino acids are possible causes of reduced growth in this study (Tiamiyu *et al*. 2016; Osuigwe *et al*. 2002). The tolerant of *C. gariepinus* to *C. ensiformis* diet upto 50% inclusion level was due to processing method, dietary inclusions levels, test organism and nature of other feed ingredient used. The result in this study was higher than that of Tiamiyu *et al.* 2016; Fagbenro *et al*. 2004; Martinez-Palacios *et al*. 1998; Akinbiyi, 1992 and Abdo de la Parra *et al*. 1998. Despite depressed growth recorded with increasing levels of toasted *C. ensiformis*, mortality of fish was not significantly affected at lower inclusion levels, but at higher inclusion levels (75%-100%). This is in agreement with the observations made by Martinez-Palacios *et al*. (1998) who reported significant mortality for *O. mossambicus* fry fed *C. ensiformis* seed meal. However, from the result of this study it can be concluded that the detrimental effect of feeding *C. ensiformis* meal was not lethal at 50% replacement for soybeans meal.

The ability of fish to convert feeds to flesh is usually assessed in fish nutritional study is by determining the FCR and PER. Adeniyi and lawal (2017) reported that the lower the value of FCR and higher the PER, the better for the farmer. Specific growth rate was highest in the control while in terms of *C. ensiformis* diets, 25% and 50% TCe diets were higher. Osuigwe *et al*. 2002 and Olvera *et al*. 1988 observed the same trend for tilapia fed Sabania seed meal. DeSilva and Anderson (1995) reported that protein efficiency ratio is a measurement of protein effectiveness to provide the essential amino acids needed by the fish. They reported that this index has been associated with fat deposition in fish muscle. It means that higher protein efficiency ratio is an indication of diet that can produce fatty fish. The values for protein efficiency ratio showed the same pattern as specific growth rate, protein intake, feed intake, feed conversion ratio and correspond with the report of Osuigwe *et al*. 2002.

The condition factor at the end of the experiment showed that the feeds were properly utilized for better growth and health in all the treatments, since all the condition factors for the treatments were above 1.0 and correspond with the report of Sogbesan, 2007. Condition factor even in the wild is not constant for individuals, species or population but is subject to wide variations. For fish, Adiku, 2003 recorded 1.0 as the best natural condition factor and the result from this study reported higher

value. The reduction in K_2 might be due to changing of environment from hatchery condition to laboratory condition, imported feed to local feed, extraneous factor and as fish grow bigger the condition factor reduces (Sogbesan *et al*. 2017).

Histopathological Effect of Feeding Toasted *Canavalia ensiformis* Diets to *Clarias gariepinus*

The significantly ($p<0.001$) high liver (LIS), Heart (HIS), Kidney (KSI) and Gill (GSI) indices in fish fed control diets compared to the TCe diets indicated that these organs reduction in size could have been as result of deposition of toxic substance or antinutrients (Adesina, 2017). The increase in LSI value in an ideal environment is related to normal liver growth but, in cases of pollution, liver enlargement is associated with hyperplasia (Hoque *et al*. 1998; Adesina, 2017; Sogbesan, 2007 and Adejinmi, 2000). The HSI observed in the fish fed 0% TCe-based diet (control diet) indicated normal liver growth resulting from dietary treatment without TCe seed meal at this level. On the contrary, Adejinmi, 2000 noted that enlargement of organs such as liver, kidney and heart has been associated with dietary factors especially if diets contain toxins, antinutrients or heavy metals. Akerman *et al*. 2003 also found a decrease in HSI values after nine weeks in rainbow trout, *Oncorhynchus mykiss*, injected with paraquat.

Evaluation of histological structure of digestive organs in fish fed new dietary ingredients generates valuable information about their digestive capacity and the potential health effects of such diets (Diaz *et al.* 2006). Incorporating different inclusion levels of toasted *Canavalia ensiformis* seed meal (TCeM) in the diets of *C. gariepinus* in this study caused varying degrees of histopathological changes in their liver cells (hepatocytes) such as mild and severe diffuse vacuolations of the hepatocytes, vacuolar degeneration, etc. These observations agree with those of Uwachukwu *et al.* 2003 who reported that diets containing raw beans caused extensive periportal necrosis with some mononuclear cell infiltration in the livers of broilers while the centrilobular areas showed vacuolation and degeneration of hepatocytes. Olasunkanmi, 2015 also observed that *C. gariepinus* fed processed velvet beans showed both mild diffuse and marked widespread vacuolation of the hepatocytes. Vacuolated hepatocytes are usually accumulated with glycogen and have little or no regenerative ability (Nayak *et al*. 199996) and the excessive vacuolation of the hepatocytes would result in abnormal functioning of the liver cells, for example, immobilization of fat, which could consequently result in fatty infiltration of the hepatic parenchyma (Adeyemo, 2005). The result of the present study closely supports the finding of Hlophe and Moyo 2005 who observed that *C. gariepinus* fed high moringa leaf meal inclusion levels (>50%) showed an increase in the number of degraded irregularly shaped hepatocytes, small dark pyknotic nuclei, poor fatty deposition and isolated necrosis. Despite similar protein and energy levels in the experimental diets, liver histology showed that fish fed higher BSSM inclusion levels had necrotic signs associated with poor nutritional status (Tusche *et al*. 2014). In the present study, the lesions observed in the liver might probably have resulted from the excessive work load done by the liver of the experimental fish during the processes of detoxification and removal of toxicants from its body. In this situation, anti-nutritional substances present in sunflower seed meal must have been responsible for the observed histopathological changes in the liver sections.

Most common alterations found in the kidneys of fishes are tubule degeneration, dilation of Capillaries in the glomerulus and reduction of Bowman's capsular space (Takashima and Hibya 1995). The presence of tubule disruption and necrosis in the kidney of fish fed higher TCeM inclusions indicates that the kidney suffered some damage which could be attributed to the presence of anti-nutritional substances in the TCeM.

The gills are important organs for respiration, osmoregulation, acid-base balance and nitrogenous waste excretion (Hadi and Alwan 2012; Heath, 1987). It is possible that the damage of the gills could be a direct result of the salts, heavy metals, pesticides, sewage and fertilizers which are conveyed to the water and anti-nutrients from the seeds (Temmink *et al*. 1983). They are directly exposed to poisons occurring in the external environment which often cause pathology in fish (Mallatt, 1985).

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