



Growth performance and histological response of *Clarias gariepinus* raised on unprocessed dried leaves of *Telfairia occidentalis* and *Mucuna utilis*

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ARTICLE INFO

Keywords:

Clinical signs
Fish meal
Inclusions
Nutrient utilization
Standard procedures

Received: 6 May 2022

Accepted: 18 July 2022

Available online: 20 December 2022

DOI: 10.13170/ajas.7.2.25836

ABSTRACT

Nutrient utilization is determined by absorbable and digestible nutrients in fish feeds. The aim of the experiment was to investigate the effects of unprocessed dried leaves of *Telfairia occidentalis* (UDLTO) and *Mucuna utilis* (UDLMU) on histology and growth of *C. gariepinus*. Sixteen weeks feeding trial experiment was conducted using two hundred and twenty-five *Clarias gariepinus* with a mean weight 4.16 ± 0.09 g. Fish meal was replaced with 0, 25, 50, 75 and 100% levels of UDLMU and UDLTO. Growth parameters and histological examinations were carried out according to standard procedures. Mean Weight Gain (MWG) of 0% (15.8g) was significantly higher $P < 0.05$ compared to other treatments. Specific Growth Rate (SGR) of 0% (0.64) varied significantly $P < 0.05$ than other levels of inclusions. Kidneys of fish fed 25 and 50% graded levels showed patchy vacuolar degeneration of the renal tubules. Gills of fish fed 75 and 100% graded levels showed diffuse capillaries and moderate congestion of lamellar in the gills. Tissue examinations of fish fed 50, 75 and 100% graded levels exhibited clinical signs of moderate atrophy and vacuolation of hepatocytes and diffuse hepatocellular swelling and degeneration in the liver. Histological investigations revealed fragmentation and atrophy of muscle fibre in fish fed 75% graded level. There was moderate atrophy of villi of the intestine of fish fed 100% graded level. It is apparent that the increased levels of UDLMU and UDLTO could not support good growth as presumed due to the presence of anti-nutritional factors in the leaves.

Introduction

The current global status of aquaculture production shows that cost of fish feeds cannot be afforded by farmers for raising the fish needed to meet the protein intake of the world populace. Studies have shown that fish larva can be raised by replacing fish meal with poultry by-products, soya bean meal, *Moringa oleifera* leaf meal, plant ingredients, insect ingredients, terrestrial animal by-products, microbial ingredients and genetically modified ingredients (Sales, 2009; Soltan, 2009; Mahboob, 2014; Bandara, 2018; Hussain *et al.*, 2018; El-Naggar *et al.*, 2021). Research carried out by Tuleun (2008) showed impaired performance in layers using raw *Mucuna* bean. Dauda *et al.* (2021) investigated the performance and carcass

characteristics of Rabbits fed *M. utilis* leaf meal diet. The authors concluded that rabbits can be fed with diets containing *M. utilis* leaf meal up to 20% in their diets without any detrimental effect on the performance and carcass characteristic of rabbits. Samiento-Franco *et al.* (2019), reported that 25% of *Mucuna pruriens* soaked in Calcium hydroxide can be used as an ingredient in broiler diets. Tuan *et al.* (2020) documented that *M. pruriens* beans can replace *Vicia faba* beans in common carp (*Cyprinus carpio*) production. Research carried out by Woke *et al.* (2013) has shown that an unfermented *Mucuna* diet can conveniently replace Soya bean-based diet for feeding *Oreochromis niloticus*. *Telfairia occidentalis* has been used as a feed additive in African catfish, *C. gariepinus*, the result suggested that dietary

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supplementation with *T. occidentalis* leaf powder improved the growth, feed utilization, and survival of *C. gariepinus* fingerlings. Dada and Ejete-Iroh (2016) revealed in their study that *T. occidentalis* leaf extract powder promote growth and improve gonadosomatic index and reproductive indices of female *C. gariepinus*. This study was aimed to investigate the effects of unprocessed dried leaves of *T. occidentalis* and *M. utilis* in raising fish seeds in fish hatchery and its impacts on histology and growth performance of *C. gariepinus*.

Table 1. Experimental diets formulated from different graded levels of UDLTO and UDLMU

Feed Ingredients (g)	0 %	25 %	50 %	75 %	100 %
Maize	42.2	42.2	42.2	42.2	42.2
Wheat offal	5.03	5.03	5.03	5.03	5.03
Fish meal	28.0	21.0	14.0	7.0	-
Soya bean meal	10.2	10.2	10.2	10.2	10.2
Groundnut cake	8.0	8.0	8.0	8.0	8.0
<i>M. utilis</i>	-	3.5	7.0	10.5	14.0
<i>T. occidentalis</i>	-	3.5	7.0	10.5	14.0
Salt	0.05	0.05	0.05	0.05	0.05
Bone meal	0.05	0.05	0.05	0.05	0.05
Oyster shell	0.05	0.05	0.05	0.05	0.05
Lysine	0.02	0.02	0.02	0.02	0.02
Methionine	0.02	0.02	0.02	0.02	0.02
Cassava flour	5.0	5.0	5.0	5.0	5.0
Total	100.0	100.0	100.0	100.0	100.0

Materials and Methods

Study site

The experiment was carried out at Animal Genetic Resources Department (Fishery Section) of National Centre for Genetic Resources and Biotechnology (NACGRAB), Moor Plantation, Ibadan, Oyo State, Nigeria.

Fish sample collection

Two hundred and twenty-five *C. gariepinus* with mean weight of 4.16 ± 0.04 g were purchased from a reputable fish farm in Ibadan. The fish were transported to the laboratory inside fifty liters experimental container. Acclimation of fish samples took place in the laboratory at NAGRABB for two weeks.

Collection of *T. occidentalis* and *M. utilis* leaves

Leaves of *T. occidentalis* and *M. utilis* were collected from the Animal Genetic Resources department (Fishery section), NACGRAB, Moor plantation, Ibadan. *M. utilis* and *T. occidentalis* leaves were identified and authenticated in the

herbarium unit of NACGRAB. After authentication, the leaves were cleaned of dirt and air-dried at room temperature for two weeks. The two dried leaves were grounded into powder using a kitchen blender and kept inside a tight container.

Formulation of experimental diet

Telfairia occidentalis and *M. utilis* leaves were grounded and used to compound experimental diets for the fish (Table 1). Feed ingredients (maize, wheat offal, fish meal, soya bean meal, groundnut cake, cassava flour, oyster shell, bone meal, lysine, methionine and salt) were mixed with the grounded leaves of UDLMU and UDLTO. These mixtures were moistened with warm water and were pelletized into 2 mm fish pellets using a locally fabricated pelletizing machine. Pellets were sun-dried and stored inside an air tight container.

Experimental design

Fish samples were stocked at fifteen fish per treatment and replicated thrice (R_1 , R_2 and R_3) using completely randomized design. The experimental diets were graded at different levels (0 %, 25 %, 50 %, 75 % and 100 %). Fish samples were weighed every two weeks using top-loading sensitive weighing balance. Experimental diets were fed to the fish at five percent body weight. New weight obtained at the end of two weeks was used to determine the quantity of experimental diets fed to the fish.

Growth parameters

At the end of the feeding trial experiment, growth parameters such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated.

- MWG: MWG of the fish samples in each treatment was calculated according to the method described by Ishwata (1969):

$$\bar{Y}WG = \bar{Y}W_2 - \bar{Y}W_1$$

where; $\bar{Y}WG$ = Mean weight gain; $\bar{Y}W_2$ = Final mean weight gain; $\bar{Y}W_1$ = Initial mean weight gain.

- SGR: SGR of each treatment was determined as described by Brown (1957):

$$SGR = \frac{(\ln W_2 - \ln W_1)}{T_2 - T_1} \times 100$$

where; SGR = Specific Growth Rate; $\bar{Y}W_2$ = Final Mean Weight; $\bar{Y}W_1$ = Initial Mean Weight; T_2 = Final day of feeding trial experiment; T_1 = Initial day of feeding trial experiment.

- FCR: FCR was calculated as described by Hephher (1988):

$$FCR = \frac{\text{Live weight gain}}{\text{Feed intake}}$$

d. PER = PER for each treatment was calculated according to the formula described by Zeitoun et al. (1973):

$$PER = \frac{\text{Weight gain}}{\text{Protein Intake}}$$

Proximate analysis

Proximate compositions (crude protein, crude fibre, ether extract, nitrogen free extract, ash, fat, moisture content) of *T. occidentalis* and *M. utilis* leaves were determined according to the procedures described by AOAC (2005).

Phytochemical Screening of UDLTO and UDLMU

Phytochemical screening of UDLTO and UDLMU were carried out as described by Harborne (1973) and Trease and Evans (1989).

Histological examination of vital organs

At the end of the feeding trial experiment, fish samples were collected from each treatment and anaesthetized with Benzocaine. Fish were dissected and vital organs such as gills, liver, intestine, heart and kidneys were removed. Histological examinations of the vital organs were carried out according to standard procedures described by Delahoy et al. (2018). The vital organs were fixed inside 4% formaldehyde (Lach-Ner, Czech Republic). Tissues of the organs dissected were stained with Hematoxylin and Eosin. Microphotographs of the slides of the tissues of the organs were taken using a Leica DM LS microscope

(Leica, Austria) with a light microscope (U-MDOB, Olympus optical Co. Ltd., Japan).

Statistical analysis

The data were analyzed using a one-way analysis of variance, (ANOVA) at 5% level of significance. Post-hoc comparison of the significance of variance results obtained from ANOVA was done using LSD (Least significant difference) tests.

Table 2. Proximate Compositions of UDLTO and UDLMU.

Parameters	UDLTO	UDLMU
Crude protein (%)	30.05±0.01	26.78±0.09
Crude fat (%)	9.85±0.02	3.70±0.02
Crude fibre (%)	2.61±0.11	14.11±0.03
Ash (%)	13.34±0.08	8.76±0.03
Moisture (%)	5.67±0.02	7.23±0.02
Dry matter (%)	94.33±0.13	92.77±0.02
Carbohydrate (%)	38.50±0.22	39.42±0.01

Table 3. Phytochemical properties of UDLTO and UDLMU.

Parameters	UDLTO	UDLMU
Flavonoids (%)	1.22±1.11	7.60±1.35
Alkaloids (%)	0.10±0.45	11.23±0.78
Saponins (%)	0.18±0.65	28.56±0.65
Tannins(%)	3.23±0.19	30.23±0.03
Hydrogen cyanides (mg/kg)	7.78±0.09	18.59±0.22
Phenols(%)	0.34±1.56	6.78±0.02

Table 4. Water quality parameters.

Parameters	(0 %)	(25 %)	(50 %)	(75 %)	(100 %)
pH	5.52±0.10	5.87±0.11	5.69±1.12	5.90±0.98	5.45±0.86
DO (mg/l)	6.55±1.23	6.80±1.89	6.89±1.26	7.40±1.45	7.68±1.58
Ammonia (mg/l)	0.01 ^b ±0.99	0.01 ^b ±0.84	0.02 ^b ±0.22	0.05 ^a ±0.67	0.07 ^a ±0.44
Nitrite (mg/l)	0.01 ^b ±1.38	0.02 ^b ±1.25	0.02 ^b ±1.66	0.04 ^a ±1.25	0.05 ^a ±1.78

Means with the same superscripts are not significant at 5% level while those with different superscript are significant (P> 0.05)

Table 5. Growth Performance of *C. gariepinus* fed different levels of UDLTO and UDLMU.

Growth Parameters	0 %	25 %	50 %	75 %	100%
MIW (g)	3.8	4.1	4.1	4.4	4.4
MFW (g)	19.6	13.9	12.3	12.2	9.6
MWG (g)	15.8 ^a	9.8 ^b	8.2 ^c	7.8 ^c	5.2 ^d
SGR	2.49 ^a	2.06 ^b	1.90 ^c	1.85 ^c	1.49 ^d
FCR	0.74 ^a	0.43 ^b	0.36 ^c	0.32 ^c	0.21 ^d

Means with the same superscripts along the row are not significant at 5% level of significance (p>0.05).

Results

The total number of domestic pigeons was estimated to be 100, and the prevalence of infected coccidian was 72%. *Eimeria* spp. The oocysts were isolated in the test material was *E. labbeana*.

Proximate compositions of UDLTO and UDLMU

Proximate compositions of UDLTO and UDLMU are presented in Table 2. The results revealed that UDLMU contained crude protein (26.78 ± 0.09), crude fat (3.70 ± 0.02), crude fibre (14.11 ± 0.03), ash (8.76 ± 0.03), moisture (7.23 ± 0.02), dry matter (92.77 ± 0.02) and carbohydrate (39.42 ± 0.01), while UDLTO had crude protein (30.05 ± 0.01), crude fat (9.85 ± 0.02), crude fibre (2.61 ± 0.11), ash (13.34 ± 0.08), moisture (5.67 ± 0.02), dry matter (94.33 ± 0.13) and carbohydrate (38.50 ± 0.22).

Water quality monitoring

Water quality parameters of the water media are presented in Table 4. All the water quality parameters recorded in this study showed no level of significance and were within the range required for the culture of freshwater fishes.

Table 5 shows the growth performance and nutrient utilization of UDLMU and UDLTO fed to *C. gariepinus*. MWG of 0% (15.8g) was significantly higher $P < 0.05$ compared to other treatments. Likewise, the SGR of 0% (0.64) varied significantly $P < 0.05$ than other levels of inclusion. FCR of 100% (0.21) was significantly lower than other treatments.

Cross-sections of vital organs of *C. gariepinus* affected by different graded levels of UDLTO and UDLTMU are shown in Figure 1 to Figure 9. At 25% level of UDLTO and UDLMU fed to *C. gariepinus*, all the organs were not affected by the level of inclusion except the kidney which showed patchy vacuolar degeneration of the renal tubules (Figure 1). Likewise, in fish fed 50% level of inclusion, only the kidney showed patchy vacuolar degeneration of the renal tubules as found in the kidney of fish fed 25% level of inclusion (Figure 2). Tissue examination of fish fed 50% UDLTO and UDLMU revealed that there was moderate atrophy of hepatocytes in the liver (Figure 3). Gills of *C. gariepinus* fed 75% graded level of UDLMU and UDLTO showed diffused capillaries (Figure 4). At 75% level of UDLTO and UDLMU, there was diffused hepatocellular swelling and degeneration in the liver of *C. gariepinus* (Figure 5). Fragmentation and atrophy of muscle fibre occurred in fish fed 75% graded levels of UDLTO and UDLMU (Figure 6).

Section of tissues of gills of fish fed 100% graded level of UDLTO and UDLMU exhibited clinical signs of moderate congestion of lamellar in the gills examined (Figure 7). There was moderate atrophy of villi of intestine of fish fed 100% graded level of UDLTO and UDLMU (Figure 8). The liver of fish fed 100% graded level of UDLTO and UDLMU showed sign of moderate vacuolation of hepatocytes (Figure 9).

Discussion

In this study proximate composition analysis showed that dry matter (94.33%), crude protein (30.05%), crude fat (9.85%) and ash (13.35%) were higher than the proximate analysis of fluted pumpkin (*Telfairia occidentalis*) pod determined by Akwukwaegbu et al. (2016). Phytochemicals screening revealed lower values (tannins 3.23 ± 0.19), saponins 0.18 ± 0.65 , alkaloids 0.10 ± 0.45 , flavonoids 1.22 ± 1.11 and phenol 0.34 ± 1.56) compared with Usunobun and Egharebva (2014) findings in phytochemical analysis of *T. occidentalis* aqueous leaf extract. The disparity in the values obtained in this study and other authors could be attributed to the plant species, method of harvesting, handling, storage, environmental and climatic factors of where the plants are located. It was observed in this study that experimental fish had a decrease in growth as the graded levels of UDLMU and UDLTO increased in the diet. The high level of phytochemicals especially tannins and saponins as presented in Table 3 may be responsible for the observed decreased in fish growth. It was very definite from this finding that tannins and saponins might have bonded with proteins or amino acids in the experimental diets. Bonding formation might have made the nutrients indigestible due to nutrient interaction. This assertion agrees with the findings of Aleator (1993) who reported that a high level of tannin in the diet of rats, chicks and livestock adversely affected the digestibility of proteins and carbohydrates, thereby reducing fish growth and availability of amino acids to the fish. Turner et al. (2002) reported phytic acids to have a negative effect on amino acid digestibility, thereby causing problems to non-ruminant animals due to insufficient phytase needed for hydrolysis of phytic acids. Similarly, Ajiboye et al. (2017) and Ajiboye et al. (2019) reported poor growth performance in *C. gariepinus* as the level of dried poultry droppings and dried pig feces increased in the diets of the fish. The authors attributed poor growth performance recorded at 100% level of inclusion

to the high level of microbes and phytochemicals in the formulated diets.

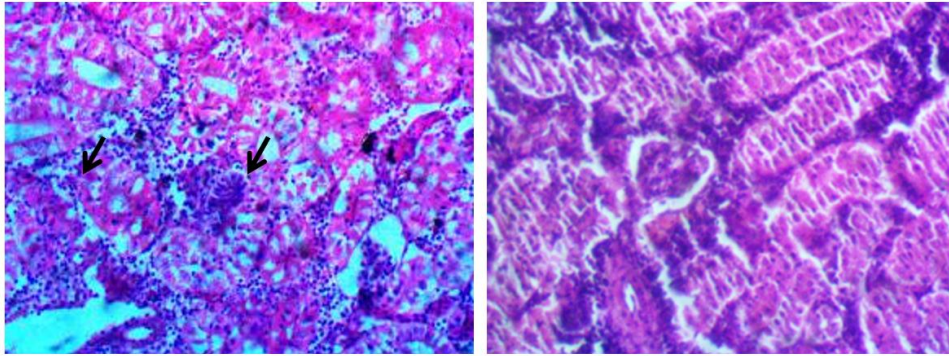


Figure 1. Cross sections of kidney of *C. gariepinus* fed 25 and 0% UDLTO and UDLMU showing patchy vacuolar degeneration of the renal tubules (arrows). HE x400.

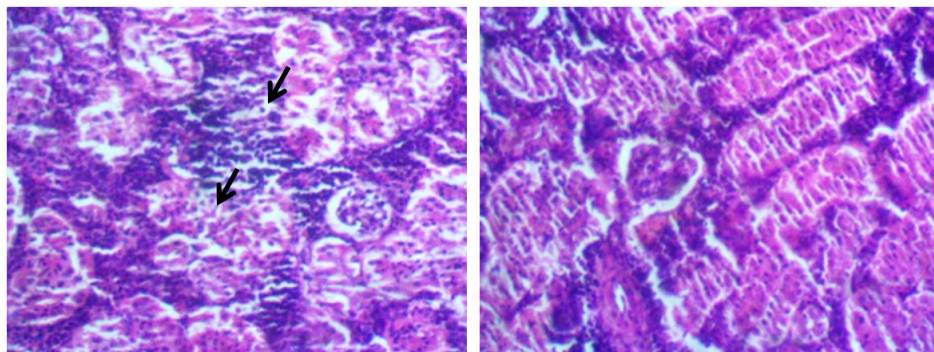


Figure 2. Cross sections of kidney of *C. gariepinus* fed 50 and 0% UDLTO and UDLMU showing patchy vacuolar degeneration of the renal tubules (arrows). HE x400.

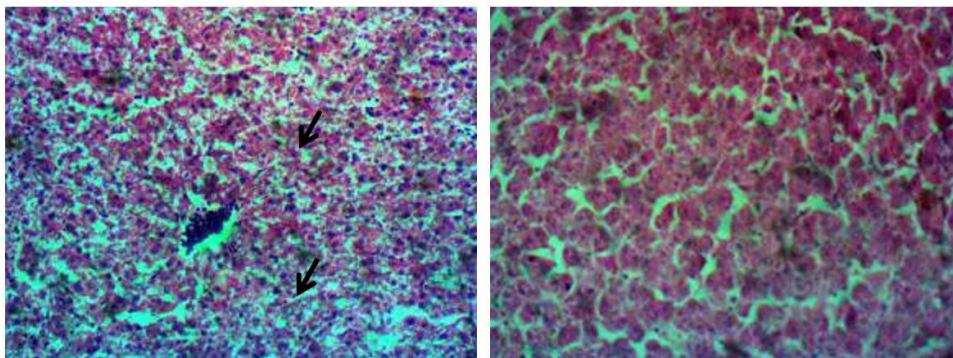


Figure 3. Cross sections of liver of *C. gariepinus* fed 50 and 0% UDLTO and UDLMU showing moderate atrophy of hepatocytes (arrows). HE x400.

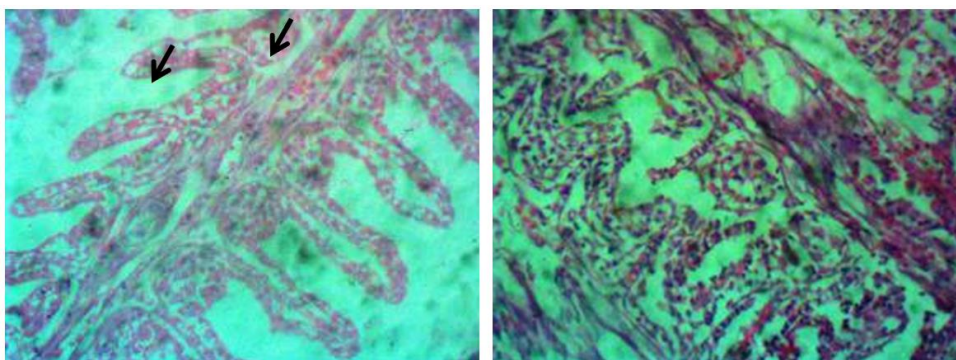


Figure 4. Cross sections of gills of *C. gariepinus* fed 75 and 0% UDLTO and UDLMU showing diffusely congested capillaries (arrows). HE x400.

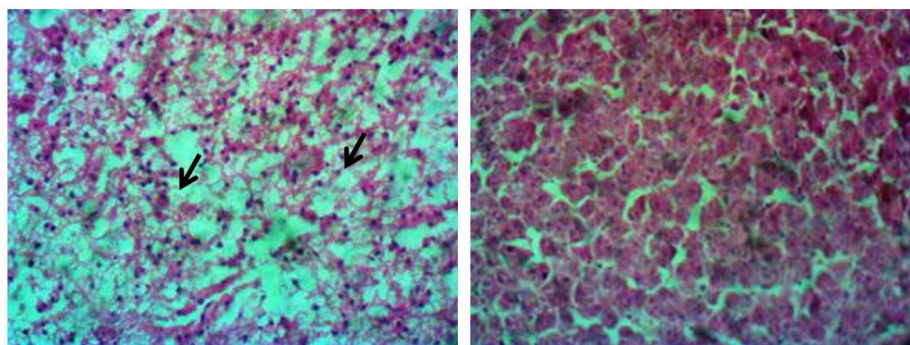


Figure 5. Cross sections of liver of *C. gariepinus* fed 75 and 0% UDLTO and UDLMU showing diffuse hepatocellular swelling and degeneration (arrows). HE x400.

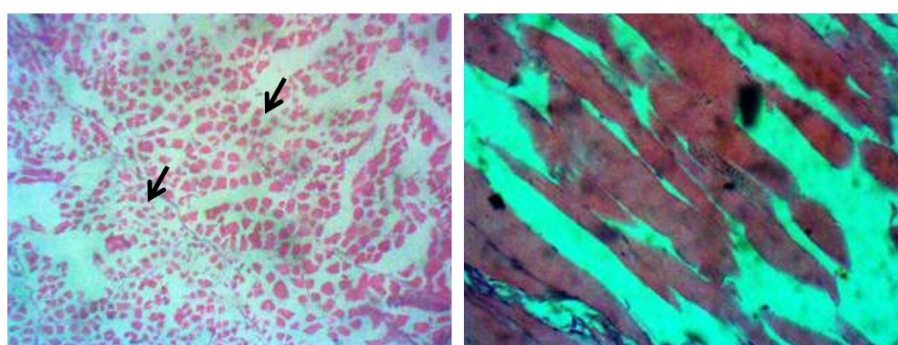


Figure 6. Cross sections of muscle of *C. gariepinus* fed 75 and 0% UDLTO and UDLMU showing fragmentation and atrophy of muscle fibre (arrows). HE x400.

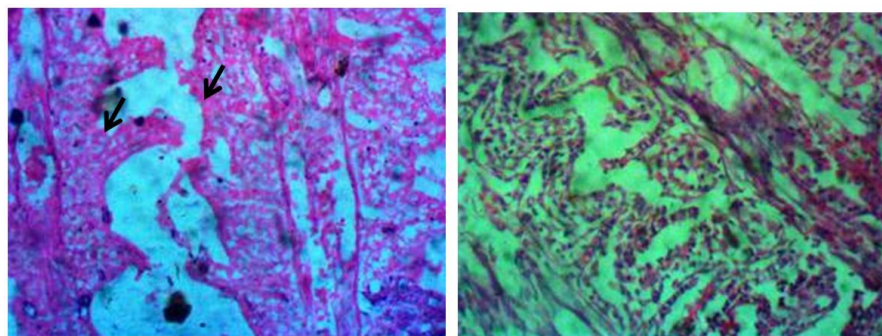


Figure 7. Cross sections of gill of *C. gariepinus* fed 100 and 0% UDLTO and UDLMU showing moderate congestion of lamellar capillaries (arrows) in the gills. HE x400.

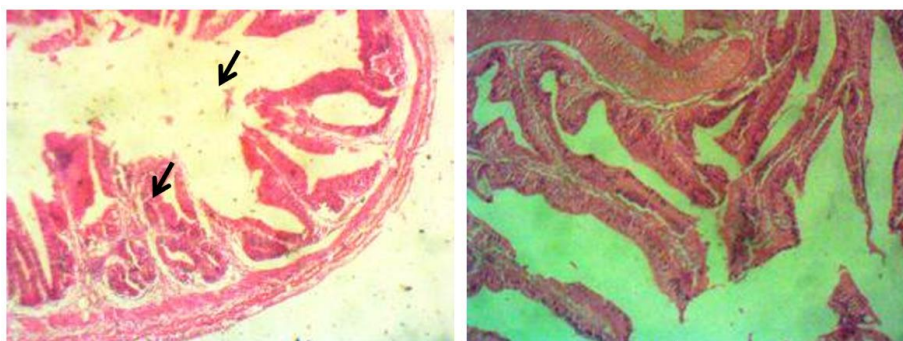


Figure 8. Cross sections of intestine of *C. gariepinus* fed 100 and 0% UDLTO and UDLMU showing moderate atrophy of villi of the intestine (arrows). HE x400.

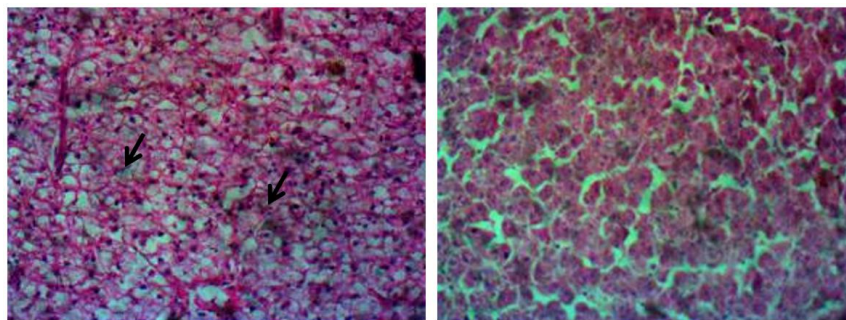


Figure 9. Cross sections of liver of *C. gariepinus* fed 100 and 0% UDLTO and UDLMU showing moderate vacuolation of hepatocytes (arrows). HE x400.

Tissue examinations diagnosed the kidney to have patchy vacuolar degeneration of the renal tubules. This condition of the kidney showed the death of tubular epithelial cells that formed the renal tubules of the kidneys. It was evident that a high level of phytochemicals in the inclusions might have caused the fibrosis and inflammation of the tubules. Ekanem *et al.* (2010) in their findings reported nephrotoxicity (enlarged tubules, distorted glomeruli and Bowman's capsule, shrinking of proximal and distal convoluted tubule) in Wistar rats treated orally and intraperitoneally with *T. occidentalis* root. There was moderate atrophy of villi of intestine of fish fed 100% graded level of UDLTO and UDLMU. Villi of the intestine are small finger-like projections that increase the internal surface area of the intestinal walls for absorption of nutrients from the diets fed to fish and at the same time produce the digestive secretions needed for digestion of food. This clinical signs imply size decrease or shrinkage of the villi due to poor absorption and circulation of nutrients from the diets supplied to the fish samples. Studies carried out by Kuku *et al.* (2014) reported eroded microvilli, degenerative changes in the mucosa and muscular wall in the small intestine of rats fed unprocessed fluted pumpkin seeds. with most of the microvilli eroded. The gills of fish fed 75% UDLMU and UDLTO that exhibited diffused gill capillaries suggest that the diffusion of oxygen and carbon dioxide may be difficult between the respiratory system and the bloodstream. Akinsanya *et al.* (2016) and Elahee and Bhagwant, (2007) reported hypertrophy of epithelium, tissue proliferation, congestion of blood vessel and hyperplasia of the lamella of gills of *C. gariepinus*. The authors explained that this will lead to an overall reduction in the efficiency of gill filaments which aid the diffusion of oxygen across the gill lamellae. Fragmentation and atrophy of muscle fibres were observed in this study probably due to the unavailability of nutrients caused by the high level of tannins and saponins in the diets fed at 75%

graded level. This occurrence resulted in the activation and removal of proteolytic systems and proteins thereby causing muscle fiber shrinkage. Wang *et al.* (2021) reported an imbalance of protein homeostasis, with excessive protein degradation which resulted in the development of muscle atrophy. Muscle atrophy could be caused and triggered by many factors such as inflammation, malnutrition, aging, cancer and a high concentration of fat in the diet. The liver of fish fed 100% graded level of UDLTO and UDLMU showed signs of moderate vacuolation of hepatocytes. Pathologically, the sign of vacuolation of hepatocyte implies liver injury. The high level of tannin and saponins caused the damage to the liver at 100% graded level of UDLMU. It is definite that damage to hepatocytes caused the inability of the liver to perform detoxification of anti-nutritional factor in the diets fed to the fish.

Conclusions

The findings of this study validate the presence of anti-nutritional factors such as tannins and saponins, and caused poor growth of *C. gariepinus* at higher graded levels of UDLTO and UDLMU. It was evident that dysfunction of vital organs like kidneys, liver, gills, intestine and muscle might be caused by the presence of these anti-nutritional factors. These findings suggest that the presence of anti-nutritional factors in the leaves of *M. utilis* and *T. occidentalis* was due to the unprocessed condition before they were included in the experimental diets

It is therefore recommended that alternatives feed ingredients must be subjected to phytochemical screening before they are used to formulate feed for fish. This therefore calls for processing methods for the removal of phytochemicals in plants that will be recruited for feed formulation.

References

- Abdisa, T., R. Hasen, T. Tagesu, G. Regea, G. Tadese. 2019. Poultry coccidiosis and its prevention, control. *Journal of Veterinary and Animal Research* 1, (1):1-6.
- Ajiyoye, A.O., A.A. Owoseni, F. Ajani. 2017. Effects of varying levels of dried poultry droppings on haematology, microbial properties and nutrient utilization of *Clarias gariepinus*. *Notulae Scientia Biologicae*, 9(4): 460-465.
- Ajiyoye, A.O., F. Ajani, B.O. Emikpe, A.B. Adesanya. 2019. Dried pig feces: impacts on growth, haematology and histology of *Clarias gariepinus*. *Annual Research and Review in Biology*, 32(4):1-10.
- Akinsanya, B., O.U. Utoh, U.D. Ukwu. 2016. Toxicological, phytochemical and anthelmintic properties of rich plant extracts on *Clarias gariepinus*. *The Journal of Basic and Applied Zoology*, 74: 75-86.
- Akwukwaegbu, P.I., D.E. Peters, M.O. Wegwu. 2016. Proximate analysis and phytochemical screening of fluted pumpkin (*Telfairia occidentalis*) pod. *American Journal of Food, Nutrition and Health*, 1(1):1-6.
- Aletor, V.A. 1993. Allelochemicals in plant food and feeding stuffs: 1. Nutritional, biochemical and physiopathological aspects in animal production. *Veterinary and Human Toxicology*, 35: 57-67.
- AOAC. 2005. Association of official analytical chemist. Official method of analysis, Washington, DC.
- Bandara, T. 2018. Alternative feed ingredients in aquaculture: Opportunities and challenges. *Journal of Entomology and Zoology Studies*, 6(2): 3087-3094.
- Brown, M.E. 1957. Experimental studies on growth. In: *The physiology of fishes*. Academic Press, New York, 1:361-400.
- Dada, A.A., V.C. Ejete-Iroh. 2016. Dietary effects of *Telfairia occidentalis* leaf extract powder on the egg quality of African catfish (*Clarias gariepinus*) broodstock. *Journal of Aquatic Sciences*, 31(1):75-84.
- Dauda, A., A.J. Akomka, U.A. Mary, T.N. Kperun. 2021. Effects of *Mucuna utilis* leaf meal diet on performance and carcass characteristic of rabbits. *Journal of Veterinary Research Advances*, 3(1): 41-45.
- Delahoy, M.J., B. Wodnik, L. McAliley, G. Penakalapati, J. Swarthout, M.C. Freeman, K. Levy. 2018. Pathogens transmitted in animal feces in low- and middle-income countries. *International Journal of Hygiene and Environmental Health*, 221:661-676.
- Ekanem, T.B., A.O. Ekeoma, M.A. Eluwa, A.O. Akpantah. 2010. Influence of crude extract of root of *Telfairia occidentalis* (fluted pumpkin) on the cytoarchitecture of the rat kidney and body weight. *Global Journal of Pure and Applied Sciences*, 16(3): 353- 357.
- Elahee, K.B., S. Bhagwant. 2007. Hematological and gill histopathological parameters of three tropical fish species from a polluted lagoon on the west coast of Mauritius. *Ecotoxicology and Environmental Safety*, 68(3):361-371
- El-Naggar, K., R. Mohammed, M.I. El-Katcha, S.E. Abdo, M.A. Soltan. 2021. Plant Ingredient diet supplemented with lecithin as fish meal and fish oil alternative affects growth performance, serum biochemical, lipid metabolism and growth-related gene expression in Nile tilapia. *Aquaculture Research*, 52(12): 6308-6321.
- Harborne, J. B. 1973. *Phytochemical methods: A guide to modern techniques of plant analysis*. Chapman and Hall Ltd, London. pp. 279.
- Hepher, B. 1988. *Nutrition of pond fishes*. Cambridge University Press. UK. p. 388.
- Hussain, S.M., M.Z.H. Arsalan, A. Javid, A.I. Hussain, N. Aslam, Q. Ali, M. Hussain, M.M.H. Rehan, M.M. Shahzad, A. Khalid, D. Riaz. 2018. Replacement of fish meal with *Moringa oleifera* leaf meal (MOLM) and its effect on growth performance and nutrient digestibility in *Labeo rohita* fingerlings. *Pakistan Journal of Zoology*, 50(5): 1815-1823.
- Ishwata, N. 1969. Ecological studies on the feeding of fishes. Frequency of feeding and growth. *Bulletin of the Japanese Society of Scientific Fisheries*, 35: 985-989.
- Kuku, A.I., U.J. Etti, I.S. Ibironke. 2014. Processing of fluted pumpkin seeds, *Telfairia occidentalis* (Hook F) as it affects growth performance and nutrient metabolism in rats. *African Journal of Food, Agriculture, Nutrition and Development*, 14(5): 9192-9214.
- Mahboob, S. 2014. Replacing fish meal with a blend of alternative plant proteins and its effect on the growth performance of *Catla catla* and *Hypophthalmichthys molitrix*. *Pakistan Journal Zoology*, 46(3): 747-752.
- Sales, J. 2009. The effects of fish meal replacement by soyabean products on fish growth: A meta-analysis. *British Journal of Nutrition*, 102(12): 1709-1722.
- Sarmiento-Franco, L., F. Lopez-Santiz, R. Santos-Ricalde, C. Sandoval-Castro. 2019. *Mucuna pruriens* seeds given in broiler diets on growth performance and carcass yield. *Ecosistemas Recursos Agropecuarios*, 6(16): 121-127.
- Soltan, M. A. 2009. Effect of dietary fish meal replacement by poultry by-product meal with different grain source and enzyme supplementation on performance, feces recovery, body composition and nutrient balance of Nile Tilapia. *Pakistan Journal of Nutrition*, 8: 395-407.
- Trease, G.E., Evans, W.C. 1989. *Pharmacognosy*. 13th Edition, Tindall, London.
- Tuan, N.N., T.D. Hoai, S. Yaemkong. 2020. Replacement of velvet bean (*Mucuna pruriens*) with faba bean (*Vicia faba*) in crisp common carp (*Cyprinus carpio*) production. *AACL Bioflux*, 13(2): 1055-1063.
- Tuleun, C.D., S.N. Carew, I. Ajiyoye. 2008. Feeding value of velvet beans (*Mucuna utilis*) for laying hens. *Livestock Research for Rural Development*, 20(5): 81.
- Turner, B.L., J.M. Paphazy, M.P. Haygarth, D.I. Mckelvie. 2002. Inositol phosphate in the environment. *The Royal Society*, 357: 469-449.
- Usunobun, U., E. Egharebva. 2014. Phytochemical analysis, proximate and mineral composition and in vitro antioxidant activities in *Telfairia occidentalis* aqueous leaf extract. *Journal of Basic and Applied Sciences*, 1(1): 74-87.
- Wang, Y., Q. Liu, H. Quan, S.G. Kang, K. Huang, T. Tong. 2021. Nutraceuticals in the Prevention and Treatment of the Muscle Atrophy. *Nutrients*, 13(6):1914.
- Woke, G.N., L.P. Aleye-Wokoma, G.W. Komi, D.O. Bekibele. 2013. Effect of fermented and unfermented mucuna bean seed on growth performance of tilapia. *Global Journal of Pure and Applied Sciences*, 19:9-15
- Zeitoun, I.H., J.E. Halver, D.E. Ulrey, P.I. Tack. 1973. Influence of salinity on protein requirements of Rainbow Trout (*Salmo gairdneri*) fingerlings. *Journal of the Fisheries Board of Canada*, 30(12): 1867-1873.