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Unprocessed Dried Leaves of *Mucuna utilis* and *Vernonia amygdalina*: Impacts on Raising Fish Seeds in Indoor Hatchery

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ABSTRACT: Sustainable and stable production of fish larvae requires diet that will enhance efficient nutrient utilization and growth performance. Sixteen weeks experiment was designed to investigate the effects of unprocessed dried leaves of *Mucuna utilis* (UDLMU) and *Vernonia amygdalina* (UDLVA) on hematology, histology and growth performance of *Clarias gariepinus*. Fish meal was replaced with UDLMU and UDLVA at different graded levels (0%, 25%, 50%, 75% and 100%). Growth parameters, hematology and histology of vital internal organs were determined according to standard procedures. Data obtained were analysed using ANOVA and Duncan multiple range test was used to separate the means. Mean weight gain (MWG) of 0% (15.00±1.11g) was significantly higher $P<0.05$ than other treatments. White blood cell (WBC) count varied significantly $P<0.05$ at 100% (14150± 9.87g/l) level of inclusion. Degeneration of tubular epithelial cells occurred in the kidneys of fish fed 75% level of UDLMU and UDLVA. Moderate atrophy of hepatic plates and diffuse swelling of hepatocytes were observed in the liver of fish fed 75% and 100% graded levels. In this study, values obtained shows that increased level of UDLMU and UDLVA and presence of anti-nutritional factors in the diets affected growth performance and dysfunction of liver and kidney of *C. gariepinus*.

Keywords: *Clarias gariepinus*, Growth performance, Hematology, Histology

Introduction

Vernonia amygdalina is a multipurpose plant that has several preventive and therapeutic potentials especially its antihelminthic properties (Oyeyemi and Oyeyemi, 2018). Traditional uses and biological activities of flower extracts and constituents of the flower of *V. amygdalina* against various ailments including bacteria had been documented by Habtamu and Melaku (2018). Ajala and Owoyemi (2016) investigated the effects of *V. amygdalina* on some biological parameters of milt of male African catfish, *C. gariepinus* and reported that inclusion levels did not have significant effects on sperm mobility, liveability and morphology. Pathania, *et al.* (2020) assessed the nutritive and medicinal properties of *Mucuna utilis* and reported that the plant is poisonous when ingested by ruminants, but considered the plant for its anticholesterolemic, anti-Parkinson, antioxidant, antidiabetic, sexual enhancing, anti-inflammatory, antimicrobial and antivenom activities. *M. utilis* covers various treatment models used in studying the Parkinson's disease like *Drosophilia melanogaster*, zebrafish, mice, rat and humans (Suryawanshi *et al.*, 2020). Research carried out by Dahouda *et al.* (2009) had shown that processed seeds of *M. utilis* can replace soyabean up to 20% for extensive guinea fowl production.
There is urgent need to replace some fish feed ingredients with alternative ingredients so as to sustain maximum feed production and make it affordable to all farmers. Nutritionally, fish feed ingredients determine the

digestibility, nutrient utilization, growth performance, survival and well-being of the fish. In an effort to cultivate more plant materials to replace essential and expensive feed ingredients such as fish meal, there is need for every expert in the area of fish feed formulation, processing and milling to take into cognizance that some of these ingredients contain some anti- nutritional factors that suppresses and inhibits growth in fish. Hence, this study was designed to investigate the effects of different levels of UDLMU and UDLVA on hematology, histology, nutrient utilization and growth performance of *C. gariepinus.*

Materials and methods

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

Fish sample collection: Two hundred and twenty-five *C. gariepinus* of mean weight 4.04±2 g were purchased from a reputable fish farm in Ibadan. The fish were transported to the laboratory inside fifty litres' experimental container. They were acclimatized in the laboratory at NAGRABB for fourteen days. During the acclimation time, the fish were fed with commercial feed at 5 % body weight.

Collection of M. utilis and V. amygdalina leaves: V. amygdalina and *M. utilis* leaves were collected from Animal Genetic Resources unit, NACGRAB, Moor plantation, Ibadan. The authentication of the leaves was carried out in the herbarium unit of NACGRAB. Thereafter, the leaves were cleaned of dirt and air-dried at room temperature for two weeks. The dried leaves were grounded into powder with a blender and kept inside a tight container.

Feed formulation: The grounded leaves of *M. utilis* and *V. amygdalina* leaves were used to compound experimental diets for the fish (Table 1). The ground leaves were mixed with some feed ingredients (maize, wheat offal, fish meal, soya bean meal, groundnut cake, cassava flour, oyster shell, bone meal, lysine, methionine and salt). The mixture of the grounded leaves and feed ingredients were moistened with warm water and were pelletized into 2 mm fish pellet using locally fabricated pelletizing machine. The pellets were sun dried and stored inside a tight container.

Table 1: Formulated diets using different graded levels of UDLMU and UDLVA

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
Mucuna	-	3.5	7.0	10.5	14.0
Bitter leaf	-	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

Experimental design: The experimental design used for the experiment was completely randomized design with five different levels of diets (0 %, 25 %, 50 %, 75 % and 100 %) and three replicates (R_1, R_2 and R_3). Each of the treatment was stocked with fifteen experimental fish per replicate. The fish samples were weighed fortnightly using top-loading sensitive weighing balance. Five percent of the fish biomass was used to calculate the quantity of experimental diets to be fed to the fish. The new weight was used to determine the quantity of experimental diets to be fed to the fish at the beginning of every two weeks.

Growth parameters: Growth parameters such as Mean Weight Gain (MWG), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR) and Protein Efficiency Ratio (PER) were calculated.

a) Mean Weight Gain (MWG) for each treatment was calculated according to the method described by Ishwata (1969):

$$\overline{Y}\,WG = \overline{Y}\,W_2 - \overline{Y}\,W_1$$

where: $\overline{Y}\,WG$ = Mean weight gain, $\overline{Y}\,W_2$ = Final mean weight gain, $\overline{Y}\,W_1$ = Initial mean weight gain

b) Specific Growth Rate (SGR) was determined as described by Brown (1957):

$$SGR = 100\ \frac{(\bar{Y}\ W2 - \bar{Y}\ w1)}{t\ (in\ days)}$$

where; SGR = Specific Growth Rate

$\bar{Y}\ W_2$ = Final Mean Weight;

$\bar{Y}\ W_1$ = Initial Mean Weight

t = Time in days.

c) Feed Conversion Ratio (FCR) was determined as described by Hepher (1988):

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

d) Protein Efficiency Ratio (PER) 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 the leaves of *M. utilis* and *V. amygdalina* were determined according to the procedures described by AOAC (2005)

Collection of blood samples: Blood samples of fish fed UDLMU and UDLVA were collected at the end of feeding trial experiment. Each blood collection was completed within five minutes of fish removal from the culture system. 5 ml samples were drawn from the posterior caudal vein according to Schmitt *et al.* (1999) with hypodermic needle and was decanted into plastic tubes containing the sodium salt of ethylenediaminetetraaceticacid (Na-EDTA) as an anticoagulant.

Hematological analysis: RBC, WBC and platelet counts were done using the Neubauer haemocytometer. The haematocrit or PCV and Hb concentration values were determined by the microhaematocrit capillary tube and cyanomethaemoglobin methods (Hesser, 1960), respectively. WBC and platelet were counted until 200 WBC were enumerated on blood smears and the percentage of each WBC and of platelet were multiplied by the total WBC and platelet count to obtain absolute differential cell counts. Mean Corpuscular haemoglobin concentration (MCHC), Mean Corpuscular haemoglobin (MCH) and Mean Corpuscular Volume (MCV) were calculated respectively using standard formula described by Dacie and Lewis (2016).

Determination of aspartate aminotransferase (AST): 0.5ml of the substrate, sodium azide was added in a test tube plus 0.5ml of the blood. It was then put in a water bath at 37 °C for 30 min. After this 0.5 ml of a 2,4 dinitrophenylhydrazine was added to the mixture, incubated for 20 min and 5 ml of sodium hydroxide was added to the mixture which turned brown. It was then placed in a spectrophotometer at 540 nm and the results were read on the calibrated graph.

Determination of alanine aminotransferase (ALT): 0.5 ml of substrate was added into a test tube containing 0.5 ml of blood, the mixture is put in a water bath at 37 °C for 30 min. 0.5 ml of 2,4- dinitrophenylhydrazine was added to the solution and incubated for 20 min. Then 5 ml of sodium hydroxide was added to mixture which turned brown and was placed in a spectrophotometer at 540nm and results were obtained from the calibrated graph. Calculation:

ALT(U/I) = change Abs/mm x 1750

Determination of alkaline phosphatase (ALP): 1ml of p-nitrophenylphosphatase was added to 1ml of the serum in a test tube, incubated for 30mins and results were obtained from the spectrophotometer at 410 nm and correlated with values on the calibrated graph to give the results. Albumin and globulin were estimated. The serum total proteins and albumin levels were determined using the methods described by Henry *et al.*, (1957).

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

Results

The results of proximate compositions of *M. utilis* and *V. amygdalina* are shown in Table 2. The recorded values of crude protein, crude fat, crude fibre, ash, moisture dry matter and carbohydrate were 26.78%, 3.70%, 14.11%, 8.76%, 7.23%, 92.77% and 39.42% (*M. utilis*), 23.58%, 3.52%, 15.72%, 9.32%, 8.07%, 91.93% and 39.80% (*V. amygdalina)* respectively.

Table 2: Proximate compositions of UDLMU and UDLVA

Parameters	UDLMU	UDLVA
Crude protein (%)	26.78±0.09	23.58±0.09
Crude fat (%)	3.70±0.02	3.52±0.02
Crude fibre (%)	14.11±0.03	15.72±0.03
Ash (%)	8.76±0.03	9.32±0.02
Moisture (%)	7.23±0.02	8.07±0.02
Dry matter (%)	92.77±0.02	91.93±0.02
Carbohydrate (%)	39.42±0.01	39.80±0.07

Qualitative and quantitative properties of UDLMU and UDLVA are presented in Table 3. The analysis revealed that UDLMU contain flavonoids (7.60±1.35%), alkaloids (11.23±0.78%), saponins (28.56±0.65%), tannins (30.23±0.03%), hydrogen cyanide (18.59±0.22 mg/kg) and phenols (6.78±0.02%). UDLVA had flavonoids (15.67±1.43%), alkaloids (7.12±0.76%), saponins (6.51±1.07%), tannins (4.32±0.78%), hydrogen cyanides (2.43±0.03 mg/kg) and phenols (5.56±.11%) respectively.

Table 3: Phytochemical screening of UDLMU and UDLVA

Parameters	UDLMU	UDLVA
Flavonoids (%)	7.60±1.35	15.67±1.43
Alkaloids (%)	11.23±0.78	7.12±0.76
Saponins (%)	28.56±0.65	6.51±1.07
Tannins(%)	30.23±0.03	4.32±0.78
Hydrogen cyanides (mg/kg)	18.59±0.22	2.34±0.03
Phenols(%)	6.78±0.02	5.56±.11

Water quality parameters values are presented in Table 4. Values recorded for all the water quality parameters determined in this study showed no level of significance.

Table 4: Water quality parameters

Parameters	(0 %)	(25 %)	(50 %)	(75 %)	(100 %)
pH	6.18±0.10	6.13±0.11	5.99±1.12	5.04±0.98	5.45±0.86
DO (mg/l)	6.70±1.23	6.50±1.89	6.95±1.26	7.30±1.45	7.60±1.58
Ammonia (mg/l)	0.01±0.99	0.01±0.84	0.02±0.22	0.02±0.67	0.03±0.44
Nitrite (mg/l)	0.01±1.38	0.02±1.25	0.05±1.66	0.02±1.25	0.02±1.78

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

Growth performance and nutrient utilization of *C. gariepinus* fed different levels of UDLMU and UDLVA is presented in Table 5. It was apparent in the table that MWG increased as the level of UDLMU and UDLVA decreased across the table. This trend showed that MWG of 0% (15.00±1.11g) level of UDLMU and UDLVA was significantly higher P<0.05 than other treatments fed UDLMU and UDLVA. Similarly, SGR values showed that 0% level of inclusion had the highest value (1.33±0.09), while the least value was recorded in 100% (0.74±0.06g) level of inclusion. FCR values of the five treatments revealed 0% (0.10±0.98) having the least value. All the values recorded for the SGR, FCR and PER were not significantly different.

Table 5: Nutrient utilization and growth performance of *C. gariepinus* fed different levels of UDLMU and UDLVA

Growth Parameters	0%	25%	50%	75%	100%
MIW (g)	4.42±0.09	4.41±0.05	4.30±1.01	4.40±0.12	4.41±0.16
MFW (g)	19.42^{a}±0.13	15.31^{ab}±0.17	14.40^{ab}±0.09	13.10^{bc}±0.01	10.01^{c}±1.07
MWG (g)	15.00^{a}±1.11	10.90^{ab}±1.89	10.10^{ab}±1.65	8.70^{bc}±1.09	5.60^{c}±1.75
SGR	1.33±0.09	1.13±0.01	1.09±0.07	0.98±0.02	0.74±0.06

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

Table 6: Hematological response of *C. gariepinus* fed different levels of UDLMU and UDLVA

Parameters	0 %	25%	50%	75%	100%
PCV (%)	25^{a}±1.23	26^{a}±2.01	23^{b}±1.72	25^{a}±1.47	23^{b}±1.08
HB (g/dL)	8.7^{a}±0.51	8.6^{a}±0.21	7.4^{b}±0.67	8.6^{a}±0.35	7.3^{b}±0.23
RBC (10^{6}/l)	2.57^{b}±0.35	2.44^{c}±0.32	1.69^{d}±0.76	2.63^{a}±0.38	1.74^{d}±0.63
WBC (10^{9}/l)	12950^{c}±0.81	14000^{a}±0.14	13700^{b}±0.53	13800^{b}±0.91	14150^{a}±0.87
Plat (10^{9}/l)	98000^{d}±2.10	110000^{b}±1.01	88000^{e}±2.40	103000^{c}±3.13	122000^{a}±2.93
Lympho (%)	65^{b}±3.01	71^{a}±2.17	63^{bc}±2.06	65^{b}±3.10	55^{c}±2.53
Neutro (%)	30^{b}±1.05	22^{d}±1.02	29^{bc}±2.02	27^{c}±1.06	38^{a}±1.84
Mono (%)	2±0.1	2±0.3	2±0.1	3±0.3	3±0.2
Eosin (%)	5^{a}±0.5	5^{a}±0.3	6^{a}±0.3	4^{ab} ±0.6	4^{ab}±0.4
Baso (%)	0	0	0	1±0.1	0
MCV (fl)	97.28^{d}±1.09	106.56^{c}±0.08	136.09^{a}±0.72	95.06^{d}±0.12	132.18^{b}±1.01
MCH (pg)	33.85^{cd}±0.07	35.24^{c}±0.03	43.78^{a}±0.07	32.70^{cd}±0.04	41.95^{b}±1.02
MCHC (g/l)	3.48±0.81	3.31±0.75	3.22±1.02	3.44±0.92	3.17±0.25

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

Results obtained revealed that *C. gariepinus* fed varying levels of UDLMU and UDLVA affected the blood profile. Highest value of PCV (26.0±2.01%) was observed at 25% concentration, while the lowest values were observed at 50% and 100% inclusion levels. Hemoglobin values ranged between 7.3±0.23 g/dl and 8.7±0.51g/dl. Highest Hb was observed in the control (0% inclusion) which was significantly the same with Hb of fish exposed to 25% (8.6±0.21g/dl) and 75% (8.6±0.35 g/dl). Fish fed at 75% inclusion level had the highest RBC counts (2.63±0.38 g/l), followed by the Control (2.57±0.35 g/l) while the least count (1.69±0.76 g/l) was observed at 50% inclusion. The highest value for WBC count was obtained at 100% inclusion level (14150±9.87 g/l), which was not significantly different with the value obtained at 25% inclusion (14000± 0.14 g/l) while the lowest value was obtained from the 0% (12950±0.81 g/l). Platelet count first increased significantly (P<0.05) from 98000±2.10 g/l (0%) to 110000± 1.01 g/l at 25% inclusion level. A significant decrease was obtained at 50% inclusion level while it increased again to 103000± 3.13 g/l at 75% inclusion. The highest value (122000± 2.93g/l) was obtained at 100% inclusion level.

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Table 7: Serum biochemistry of *C. gariepinus* fed different levels of UDLMU and UDLVA

Parameters	0%	25%	50%	75%	100%
Protein(g/dl)	4.6^{bc}±1.1	5.2^{a}±1.42	4.8^{b}±1.81	4.7^{bc}±1.0	4.5^{c}±1.6
ALB	1.1^{b}±0.12	1.3^{a}±0.14	1.2^{ab}±1.10	1.2^{ab}±0.19	0.9^{c}±1.12
GLO	3.5^{bc}±1.19	3.9^{a}±1.14	3.6^{b}±0.99	3.5^{bc}±1.16	3.6^{b}±1.18
A/G (UL)	0.3±1.11	0.3±1.44	0.3±1.34	0.3±1.40	0.3±1.2
AST (U/L)	180^{b}±1.66	183^{a}±1.35	180^{b}±1.75	182^{ab}±1.56	176^{c}±1.87
ALT (U/L)	21^{b}±0.89	25^{a}±0.76	19^{c}±0.91	20^{bc}± 1.10	20^{bc}±1.01
ALP(U/L)	296^{c}±1.16	322^{a}±1.09	284^{d}±1.11	300^{b}±0.99	274^{e}±0.77
BUW	5.8^{b}±0.22	6.2^{a}±0.12	6.0^{ab}± 0.99	5.6^{c}±1.10	5.6^{c}±1.11
Creatinine	0.6^{a}±0.01	0.6^{a}±0.12	0.6^{a}±1.11	0.6^{a}±1.09	0.6^{a}±1.10

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

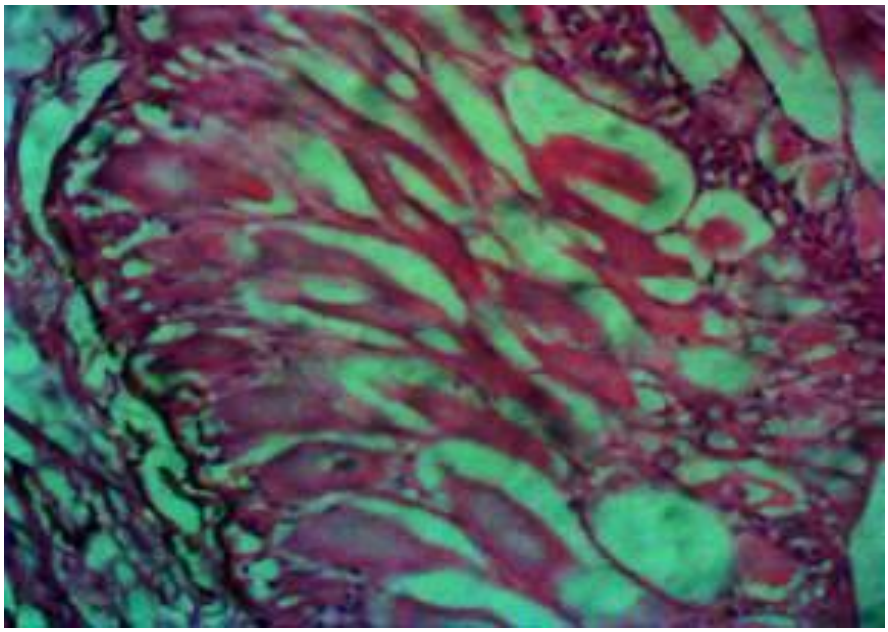

Figure 1: Cross section of skin of fish fed 100% UDLMU and UDLVA

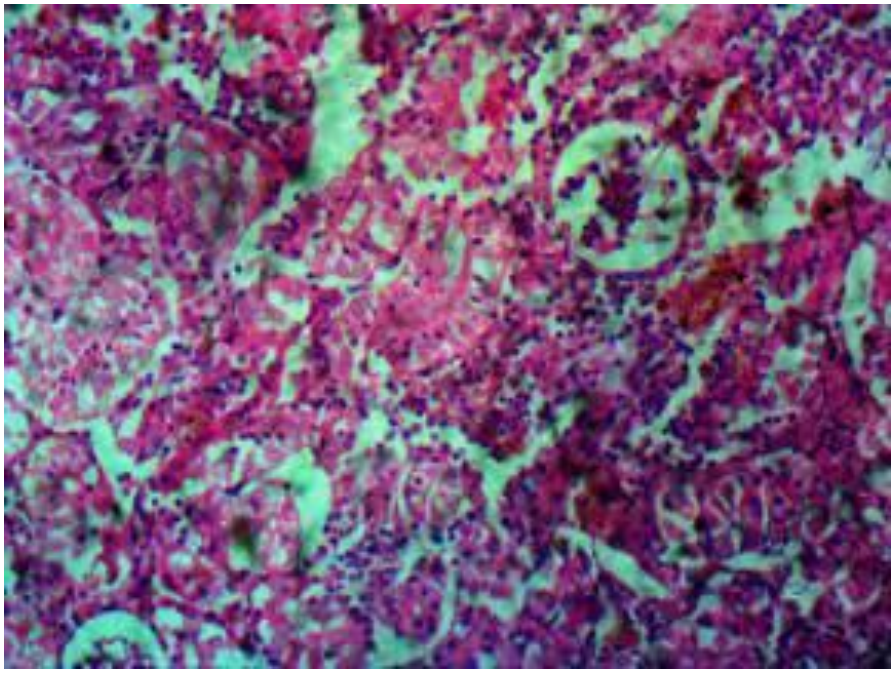

Figure 2: Cross section of kidney of fish fed 75% UDLMU and UDLVA

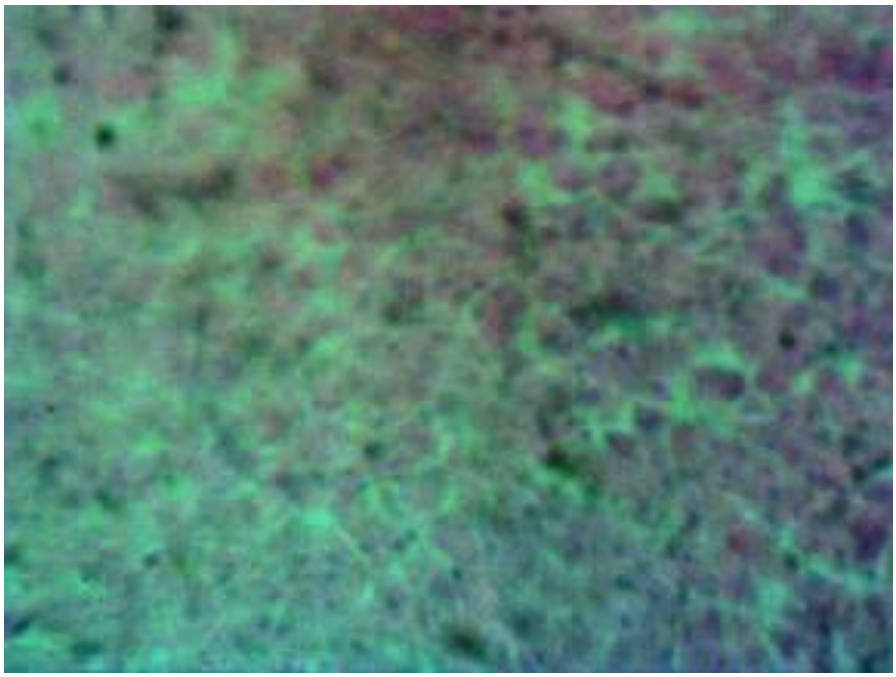

Figure 3: Cross section of liver of fish fed 75% UDLMU and UDLVA

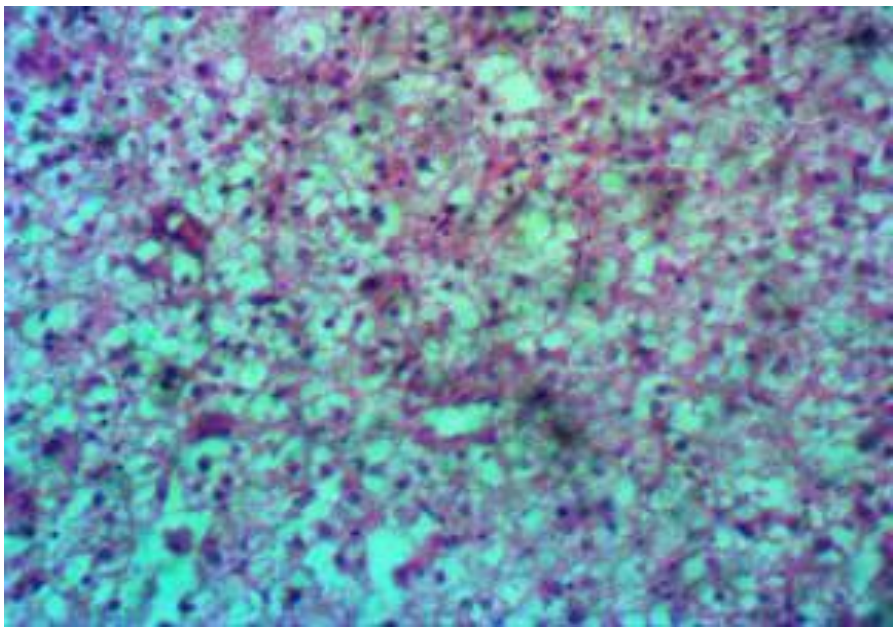

Figure 4: Cross section of liver of fish fed 100% UDLMU and UDLVA

Tissue samples of vital organs of fish affected by different levels of UDLMU and UDLVA are presented from Figure 1 to 4. Tissue samples of skin of fish fed 0%, 25%, 50% and 75% UDLMU and UDLVA showed no observable lesions. Meanwhile, there was swelling of club and epidermal cells in the skin of fish fed 100% level of inclusions of UDLMU and UDLVA (Figure 1). Histological sections of gills and intestines of fish fed 0%, 25% 50%, 75% and 100% UDLMU and UDLVA showed no observable lesions. Sections of tissues of kidneys of fish fed 0%, 25%, 50% and 100% UDLMU and UDLVA showed no observable lesions. Kidneys of fish fed 75% showed degeneration of tubular epithelial cells (Figure 2). The histological results revealed that tissues of livers of 0%, 25% and 50% showed no observable lesions. Figure 3 and 4 revealed moderate atrophy of hepatic plates in livers of fish fed 75% UDLMU and UDLVA, while moderate diffuse swelling of hepatocytes were observed in the liver of fish fed 100% level of inclusion.

Discussion

Proximate compositions of *M. utilis* and *V. amygdalina* recorded in this study showed that crude protein, ash and moisture were higher than the values recorded by Razaq (2017). Contrarily, dry matter, ether, and fibre recorded by the author were higher than the values obtained in this study. Bonsi *et al.* (1995) recorded a higher value for crude protein than the value recorded in this study. The disparities observed in these values could be attributed to the different varieties and species of the plant, method of handling the plant during and after harvesting, storage methods and mode of processing the feed ingredients. FAO (2004) postulated that type of soils for growing the plant, climatic conditions of cultured environment and geographical location of the plant could affect the proximate composition values obtained.

In this study the water quality parameters showed no level of significance and this result agreed with the findings of Ajiboye *et al.* (2015) who reported no level of significance in their study: Nutrient utilization and growth of *C. gariepinus* fed four different commercial feeds. MWG in this study increased as the level of the leaves extract decreased across the table. This indicated that growth performance was achieved at 0% level of UDLMU and UDLVA. It would be expected that *M. utilis* and *V. amygdalina* which are rich in plant protein should affect efficient nutrient utilization and growth performance. On the contrary, there was poor nutrient utilization and growth performance as the levels of *M. utilis* and *V. amygdalina* increased. Apart from proximate composition that showed the level of nutrients in feed ingredients, Ujowundu *et al.* (2010), Nwaoguikpe *et al.* (2011), Usunomena and Ngozi (2016), and Ali *et.al.* (2019) reported the presence of phytochemicals such as flavonoids, alkaloids, saponins, tannins, phenols, steroids, hydrogen cyanides in *M. utilis* and *V. amygdalina*. Phytochemical screening of *M. utilis* and *V. amygdalina* inferred that the values obtained were not conformed to the findings of Nwaoguikpe *et al.* (2011) and Ujowundu *et al.* (2010). The phytochemical properties of plants vary from one plant to the other due to different factors such as processing methods, species and varieties of the plant. These phytochemicals are known to bind with proteins in the fish diets, thereby reducing their chances of digestibility and also phenols are known to inhibit hydrolytic and digestive enzymes. 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 the diets of the fish. The authors attributed poor growth performance recorded at 100% level of inclusion to high level of microbe counts in the formulated diets with dried poultry droppings and dried pig feces. The findings in this study agreed with Fagbenro (1993), Adewumi (2014), Ajiboye *et al.* (2015b) and Ifemeje (2016), who ascertained that growth performance in fish can be affected by the level of fish meal, herbicides and anti- nutritional factor in the fish diet.

Hematological parameters, especially PCV, total and differential leukocyte counts in the blood, provide an indication of the health status of the fish (Hrubec *et al.*, 2000). A non-significant increase was first observed in the PCV of fish at 25% inclusion. Flunctuation was observed as the concentration of the inclusion increased and it finally reduced at 100% inclusion. This may be caused by red cell shrinkage as reported by Jensen *et al.*, (1987).). A decreasing trend in the values of red blood cell count, hemoglobin and hematocrit agreed with the report on rainbow trout exposed to diazinon by Banaee (2011). This may be attributed to the destruction of hematopoietic organ and adverse effect of the UDLMU and UDLVA. This trend in the result is been corroborated by Olowolafe and Olufayo (2018), when they exposed *C. gariepinus* juveniles to different levels of aqueous extracts of *V. amygdalina.* The significant increase observed in the value recorded for PCV and RBC at 75% inclusion might be attributed to the stress–mediated condition which prompts the release of new erythrocytes from the erythropoietic tissue to improve the oxygen carrying capacity of exposed fish blood with resultant higher values of erythrocyte count and hemoglobin concentration (Fafioye, 2002). Changes in WBC and differential counts have been reported to play important roles in the assessment of the state of health of *C. gariepinus* (Gabriel *et al*., 2004). Higher WBC count may explain the reason for disease resistance which has been reported by Nwosu (1979) or the prevalence of disease condition. It may also explain longevity as reported by Mbanasor *et al.* (2003). In the present study, the WBC, increased with increasing inclusion levels of UDLMU and UDLVA. This agrees with the result of Owen and Amakiri (2011) who obtained significantly higher WBC, neutrophils and lymphocytes in the *V. amygdalina* Leaf Meal as compared to the control groups. Also, Olabatoke and Oloniruha (2009) reported that *V. amygdalina* is efficient in reducing infections. Singh *et al.* (2008) also reported increased leucocyte counts of *Channa punctatus* exposed to copper. High MCH values are commonly a sign of macrocytic anemia. This condition occurs when the blood cells are too big, which can be as a result of not having enough vitamin B12 or folic acid in the body. High MCH values may also be the result of liver diseases and an overactive thyroid gland. The significant increase observed in MCV and MCH in fish fed UDLMU and UDLVA from the control to 50% inclusion may be attributed to deficiency in Vitamin B12. A high MCV and MCH indicates larger RBCs and is called macrocytosis. The fluctuations recorded for MCHC in fish exposed to UDLMU and UDLVA between 50% and 100% inclusion may be ascribed to lower concentration of haemoglobin which indicates an anaemic condition. This is corroborated by Kumar *et al.* (2013) who also observed same trend in MCH and MCHC value in infected goldfish. The MCHC is a good indicator of RBC swelling (Wepener *et al.*, 1992). Proteins are among the main energy sources which play an important role in the

maintenance of blood glucose in fish (Shwetha *et al.,* 2012). An increase in serum total protein was observed at 25% inclusion level. The main reason for increased total protein level is stress. It could mean that either albumin or globulin are high. The decrease in Serum total protein level and albumin observed in fish at 100% inclusion may be due to their degradation and also to their possible utilization for metabolic purpose. Yadav *et al.* (2003) and Bradbury *et al.* (1987) pointed out that decreased protein content and albumin might also be attributed to the destruction or necrosis of cells and consequently impairment in protein synthesis machinery. The rise in ALT and AST from 0% to 25% inclusion levels of UDLMU and UDLVA indicate use of dietary amino-acids for growth as well as compensatory for energy demand as a response to a stressor. This is also corroborated by the observation of Adesina (2008) when he worked on the toxicity of *Moringa oleifera* (Lam) extracts to *Oreochromis niloticus* fingerlings and juveniles. The observed elevated levels of ALP may indicate an increase in the rate of phosphorylation and transport of molecules across the cell membrane, which may result to increased detoxification effects of the kidney and thus a possible stress on the kidney membrane that could cause cell injury (Usese *et al.,* 2018). Similar results were however observed in *Catla catla* (Tilark *et al.,* 2002), *Labeo rohita* (Venkatesshwalu *et al.,* 2017). Albumin levels can rise due to dehydration. This is a relative increase that occurs as the volume of plasma decreases. This suggests that the exposed fish were dehydrated due to their exposure to UDLMU and UDLVA at 25% before it gradually decreased and stabilized at 50 and 75% levels of inclusion. In this study, tissue samples of gills, intestine and skin of fish examined showed no observable lesions, though swelling of clubs and epidermal cells were observed in the skin of fish fed 100% level of inclusion. Likewise, hepatic lesions were not noticeable in the livers and kidneys of fish fed 0%, 25% and 50% of UDLVA and UDLMU. These observations were recorded because the level of inclusion of UDLVA and UDLMU could not trigger any damage in the organs. Degeneration of tubular epithelial cells in the kidney, moderate atrophy of hepatic plates and diffuse swelling of hepatocytes in the liver of fish fed graded levels of 75% and 100% of UDLVA and UDLMU could be attributed to high levels of antinutritional factors present in the diets fed to the fish. Observations reported in this study were similar to hepatic lesions reported by Ajiboye *et al.* (2019) in the liver of fish fed high levels of dried pig feces. They reported severe lesions were due to detoxification of high loads of dried pig feces. Drishya *et al.* (2016) reported histopathological changes in the gills of fresh water fish, *Catla catla* exposed to electroplating effluents. The authors reported dilation and congestion in blood vessels of gill filament and atrophy of secondary lamellae. They suggested damage done to the gills could be attributed to direct result of heavy metals, salts, pesticides, fertilizers and sewage which found their ways into the water. Degeneration of tubular epithelial cell of kidney and moderate atrophy of hepatic cells and diffuse swelling of hepatocytes in the liver could be attributed to the presence of toxic compounds such as L-DOPA (3,4-L-dihydroxylphenylalanine) and hallucinogenic tryptamines and anti-nutritional factors such as phenols, tannins, saponnins and alkaloids in the UDLMU and UDLVA. The toxicity and allelopathic nature of the afore-mentined compounds and anti-nutritional factors may be responsible for why the fish are predisposed to histological dysfunctions.

Conclusion and recommendation

The findings of this study indicated that 0% level of UDLMU and UDLVA gave the best nutrient utilization and growth performance in *C. gariepinus*. Though the main objective of this study was to find alternative feed ingredients to replace vital feed ingredients especially fish meal. It was apparent that with an increase in the level of the leaves extract across the table, though expected to perform better, eventually performed poorly in terms of nutrient utilization and growth performance. The poor performance recorded in this study was due to the presence of anti-nutritional factors which inhibited digestibility and nutrient utilization in *C. gariepinus*. UDLMU and UDLVA gave negative output in terms of growth and nutrient utilization because the leaves were not processed for removal of the anti-nutritional factors. The findings of this study suggests that fish nutritionist should ensure that they investigate the presence of anti-nutritional factors in the feed ingredients before engaging them for usage for fish feed production. It is therefore expedient that further research should be carried out to investigate the impacts of processed leaves of *M. utilis* and *V. amygdalina* on nutrient utilization, growth performance, hematology and histology in *C. gariepinus*. This proposed further research will help to find the base line for food safety and enhance better aquaculture production.

References

Adesina BT: Toxicity of *Moringa oleifera* (Lam) extracts to *Oreochromis niloticus* fingerlings and juveniles. University of Ibadan, (Ph.D. Thesis). p. 261, 2008.

Adewumi AA. *Moringa oleifera* (Lam) as a protein supplement in *Clarias gariepinus* diet. Adv Res, 2(11):580 – 589. 2014.
Ajala OO, Owoyemi A: Effect of dietary *Vernonia amygdalina* del. supplementation on some biological parameters of milt of male African catfish (*Clarias gariepinus*). Bulg J Vet Med, 19(1):30-39. 2016.
Ali M, Diso SU, Waiya SA, Abdallah M: Phytochemical screening and antibacterial activity of bitter leaf (*Vernonia amygdalina*). Ann Microbiol Infect Dis, 2(4):1-7. 2019.
Ajiboye AO, Ajani F, Oyelowo OO: (2015a). Effects of oxadiazon on nutrient utilization and growth of African catfish (*Clarias gariepinus*). Am J Agric Sci, 2(3):121-125. 2015a.
Ajiboye AO, Ajani F, Folarinwa OR. (2015b). Nutrient utilization and growth of *Clarias gariepinus* fed four different commercial feeds. Int J Fish Aquac, 7(7):107-110. 2015b.
Ajiboye AO, Owoseni AA, Ajani F: Effects of varying levels of dried poultry droppings on haematology, microbial properties and nutrient utilization of *Clarias gariepinus*. Not Sci Biol, 9(4), 460-465. 2017.
Ajiboye AO, Ajani F, Emikpe BO, Adesanya AB: Dried Pig Feces: Impacts on growth, haematology and histology of *Clarias gariepinus.* Ann Res Rev Biol, 32(4):1-10. 2019.
Association of Official Analytical Chemists (AOAC): Official Methods of Analysis, 18th Edition. William Horwitz, George W. Latimer Jr. (eds). AOAC International. Washington, DC, USA. 771p. 2005.
Banaee M, Sureda ARK. Effect of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pestic Biochem Phys, 99(1):1-6, 2011. 2011.
Bonsi MLK, Osuji PO, Tuah AK, Umunna N: *Vernonia amygdalina* as a supplement of *Teffstrans eragrosticstep* fed to Ethiopian menz sheep. Agrofor Syst, 31(3):229 – 241. 1995.
Bradbury SP, Symonic DM, Coats JR, Atchism GJ: Toxicology of fenvalerete and its constituents' isomers to the fathead minnow (*Pimephales promelos*) and blue gill (*Lepomis macrochirus*). Bull Environ Contam Toxicol, 38(5):727-735. 1987.
Brown ME: Experimental studies on growth. In: The physiology of fishes. Academic Press, New York, 1:361-400. 1957.
Dacie JV, Lewis SN: Practical Haematology. 7th Edition.Churchil Livingstone, Edinburgh: Churchill Livingstone. pp 54-79. 2016.
Dahouda M, Toleba SS, Youssao AKI, Mama Ali AA, Dangou-Sapoho RK, Ahounou, SG, Hambuckers A, Hornick JL: The effects of raw and processed *Mucuna pruriens* seed based diets on the growth parameters and meat characteristics of Benin local guinea fowl (*Numida meleagris*, L). Int J Poult Sci, 8:882-889. 2009.
Drishya MK, Binu KS, Mohan KM, Ambikadevi AP, Aswin B: Histopathological changes in the gills of fresh water fish, *Catla catla* exposed to electroplating effluent. Int J Fish Aquat Stud, 4(5):13-16. 2016.
Fafioye OO. Lethal and Sublethal effects of extracts of *Parkia biglobosa* and *Raphia vinifera* on some freshwater fauna, University of Ibadan. (Ph.D. Thesis).2002.
Fagbenro OA. bservations on macadamia presscake as a supplemental feed for monosex *Tilapia guineensis* (Pisces: Cichlidae). J Aquacult Trop, **7**:91-94. 1993.
Food and Agricultural Organization of the United Nations (FAO): The State of World Fisheries and Aquaculture Production. Yearbook fisheries statistics, Food and Agricultural Organization of the United Nations, Rome, Italy.2004.
Gabriel UU, Ezeri GNO, Opabunmi OO: Influence of sex, source, health status and acclimation on the haematology of *Clarias gariepinus* (Burch, 1822). Afr J. Biotechnol, 3(9):463-467. 2004.
Habtamu A, Melaku Y: Antibacterial and antioxidant compounds from the flower extracts of *Vernomia amygdalina*. Adv Pharmacol Pharm Sci, (4):1-6. 2018.
Henry RJ, Sohel C, Berkman S: Interferences with Biuret methods for serum proteins. Anal Chem, 29(10):1491-1495. 1957.
Hepher, B: Nutrition of pond fishes. Cambridge University Press. UK, p. 388. 1988.
Hesser EF: Methods of routine fish hematology. Prog Fish Cult, 22(4):164-171. 1960.
Hrubec TC, Cardinale JL, Smith SA: Haematology and plasma chemistry reference intervals for cultured tilapia (*Oreochromis* hybrid). Vet Clin Pathol, 29(1):7–12. 2000.
Ifemeje JC: Chemical and phytochemical composition of *Mucuna pruriens* leaves. Afr J Sci Res, 5(2):14 – 17. 2016.
Ishwata N: Ecological studies on the feeding of fishes. Frequency of feeding and growth, Bull Japan Soc Sci Fish, 35:985-989. 1969.
Kumar S, Raman RP, Kumar K, Pandey PK, Kumar N, Mallesh B, Mohanty S, Kumar: Effect of azadirachtin on haematological and biochemical parameters of *Argulus*-infested goldfish *Carassius auratus* (Linn. 1758). Fish Physiol Biochem, 39(4):733–747. 2013.
Mbanasor UU, Anene BM, Chime AB, Nnaji TO, Eze JI, Ezekwe AG: Haematology of normal trypanosome infected muturu cattle in South-Eastern Nigeria. Nig J Anim Prod, 30(2):236-241. 2003.
Nwaoguikpe RN, Braide W, Ujowundu CO: The effects of processing on the proximate and phytochemical compositions of *Mucuna pruriens* seeds (Velvet Beans). Pak J Nutr, 10(10):947-951. 2011.
Nwosu CC: Characterization of the local chicken of Nigeria and its potential for egg and meat production. In: Poultry Production in Nigeria. Procedings of 1st National Seminar on Poultry Production in Nigeria, Ahmadu Bello University, Zaria,Nigeria.National Animal Production Research Institute, pp.187-210. 1979.
Olabatoke RY, Oloniruha JA. Haematological assessment of bitter leaf (*Vernonia amygdalina*) efficiency in reducing infections in cockerels. In: Proceedings of the World Congress on Medicinal and Aromatic Plants, Cape Town, South Africa, pp.472-413. 2009
Olowolafe T, Olufayo MO. Toxicity of aqueous extracts of bitter leaf (*Vernonia amygdalina)* on haematological profile of African catfish (*Clarias gariepinus*) juveniles. Int J Fish Aquat Stud, 6(2):596-600. 2018.
Owen OJ, Amakiri AO: Serological and haematological profile of broiler finishers fed graded levels of Bitter leaf (*Vernonia amygdalina*) meal. Adv Agric Biotechnol, 1:77-81. 2011.
Oyeyemi IT, Oyeyemi OT: *Vernonia amygdalina:* A folkloris herb with anthelminthic properties. Beni-Suef Univ J Basic Appl Sci, 7(1):43-49. 2018.

Pathania R, Chawla P, Khan H, Kaushik R, Khan MA: An Assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: A natural food legume. Biotechnol, 3(10): 261. 2020.

Rasaq I: Utilization of fermented *Mucuna pruriens* as replacement for soya bean meal in *Clarias gariepinus* fingerlings diets, Ahmadu Bello University, Zaria, (M. Sc. Thesis). p. 37-61. 2017.

Schmit CJ, Blazer VS, Dethloff GM, Tillitt DE, Gross TS, Bryant Jr., WL, Doweese IR, Smith Goede RW, Bortish TM, Kublak TJ: Assessing the exposure of fish to environmental contaminants. Information and Technology Report USGS/BRO-1999-0007. US. Geological Surgery, Biological Resources Division, Columbia MO, pp. 547. 1999.

Shwetha A, Hosetti BB, Dube PN: Toxic effects of zinc cyanide on some protein metabolites in fresh water fish, *Cirrhinus mrigala* (Hamilton). Int J Environ Res, 6(3):769-778. 2012.

Singh D, Nath K, Trivedi SP, Sharma YK: Impact of copper on haematological profile of fish water fish (*Channa punctatus*). J Environ Biol, 29(2):253-257. 2008.

Suryawanshi SS, Kamble PP, Bapat VA, Jadhav JP: Parkinsonism and potential of Mucuna beans, **Bioeth** Med Soc, 1-16. 2020.

Tilark KS, Yacobu K: Toxicity effects of fenvalerate on fish *Ctenopharyngodon idella*. J Ecotoxicol Environ Monit, 12(2):9-15. 2002.

Venkatesshwalu ND, Jawahra LS, Sriniva NA: Enzymatical alterations of lethal and sublethal exposure of organophosphate pesticide phorate 10% CG on *Catla catla* fingerlings. J Glob Biosci, 6(1):4666-4679. 2017.

Ujowundu CO, Kalu FN, Emejulu AA, Okafor DE, Nkwonta CG, Nwosunjoku EC: Evaluation of the chemical composition of *Mucuna utilis* leaves used in herbal medicine in Southeastern, Nigeria. Afr J Pharm Pharmacol, 4(11):811-816. 2010.

Usunomena U, Ngozi OP: Phytochemical analysis and proximate composition of *Vernonia amygdalina.* Int J Sci World, 4(1):11-14. 2016.

Usese AI, Lawal-Are AO, Moruf OR, Chukwu O.L: Biomarker responses to environmental stressors in the hairy mangrove crab, *Sesarma huzardii* (Grapsidae) from a tropical lagoon mudflat in Nigeria, Alex J Vet Sci, 57(1):4-10. 2018.

Wepener V, Van Vuren JHJ, Du Preez HH: The effect of hexavalent chromium at different pH values on the haematology of *Tilapia sparrmanii* (Chichlidae). Comp Biochem Physiol, 101(2):375–381. 1992.

Yadav RP, Singh D, Singh SK, Singh A: Metabolic changes in freshwater fish *Channa punctatus* due to stem–bark extract of *Croton tiglium***,** Pak J Biol Sci, 6(14):1223-1228. 2003.

Zeitoun IH, Halver JE, Ullrey DE, Tack PI: Influence of salinity on protein requirements of rainbow trout (*Salmo gairdneri*) fingerlings, J Fish Res Board Can, 30(12): 1867-1873. 1973.