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Blood Characteristics and Tissue Histology of *Oreochromis niloticus* Fed *Ipomoea batatas* Leaf

Diomerl Edward B. Baldo[1,5,6]*, Patricia M. Candelaria[2], Francis N. Baleta[3], Love Joy P. Baleta[4], Mylene C. Navarro[2], Lander C. Plantado[2]

[1]*Partido State University Caramoan Campus, Caramoan, Camarines Sur 4429, Philippines;* [2]*Partido State University Sagñay Campus, Sagñay, Camarines Sur 4421, Philippines;* [3]*Philippine Council for Industry, Energy and Emerging Technology Research and Development, DOST Complex, Bicutan, Taguig City 1631, Philippines;* [4]*College of Business and Management, Partido State University Goa Campus Goa, Camarines Sur, 4422, Philippines;* [5]*Biological Sciences Division, National Research Council of the Philippines, DOST Complex, Bicutan, Taguig City 1631, Philippines;* [6]*Applied Biological Sciences Program, Chulabhorn Graduate Institute, 54 Kamphaeng Phet 6 Road, Lak Si, Bangkok 10210, Thailand*

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Abstract

Clinical hematology and tissue histoarchitecture are prognostic indicators of pathological conditions in fish. Using *Oreochromis niloticus*, a 12-week feeding experiment assessed the hematology and histopathology of ammonia-exposed tissues of gills, kidney and liver. Four treatments were used in the experiment. Fish in the control group was fed the practical diet (T1), while others received diet supplemented with *Ipomoea batatas* in powder (T2), hot water extract (T3), and crude extract (T4). Hematological profiling was conducted for 12 weeks. Results indicated significant differences at week 6 in neutrophils and lymphocytes, at week 8 in erythrocytes, lymphocytes, eosinophils, and platelets, and at week 12 in hemoglobin (P<0.05). Liver of T1 and T4 illustrated histoarchitecture at normal limits, while erythrocyte infiltration and vacuolation of hepatocytes were observed in T2 and T3. All groups fed *I. batatas*-supplemented diets showed beneficial effect on the kidney as evidenced by notable decrease in the melanomacrophages. Gill tissues displayed no prominent signs of improvement against widespread infiltration of inflammatory cells in both primary and secondary lamella. The results of the present study demonstrated that incorporation of crude extracts of *I. batatas* can boost early enhancement of lymphocyte and neutrophil production and improve ammonia-induced kidney and liver histopathology.

Keywords: *Ipomoea batatas,* hematology, histology, *Oreochromis niloticus*, ammonia

1. Introduction

Aquaculture is observed to be rapidly growing in production volume and economic impact. By 2030, it is predicted to be the primary source of fish mainly due to consumption demands and reduced captured of common wild species (FAO, 2016). Expectedly, aquaculture production can feed the growing population in every part of the world. However, challenges in successful and effective expansion in aquaculture sector is faced by culture farmers (Abbas *et al.,* 2019). Important factors in this endeavor include its ability to expand sustainably, coping with changes in ecosystem, and profitable aquafeed industry (Fazio, 2019). Prices of animal protein ingredients have been increasing (Olsen and Hasan, 2012), which led to increase in the cost of fish raising, and subsequently elevated the fish product cost.

Nile tilapia (*Oreochromis niloticus)* is one of the main and accepted farmed species around the globe. While, the dietary requirement of tilapia is well reported (Wilson, 1994), alternative feed for more sustainable production, affordable sale prices and promising nutritional values is still warranted. Fish nutritionists all over the world are continuously searching for natural dietary source, which can maximize the fish production in a time-bound and at low cost manners.

Protein is now considered as the most expensive component in aquaculture diet. Thus, feed manufacturers search for alternative sources of carbohydrate and fat-rich sources (Yones *et al.,* 2019). As potential non-protein energy sources, lipids and carbohydrates are among the choices tapped in today's aquaculture development. A number of studies have shown that most cultured fish can efficiently utilize both these macromolecules for better growth (Boujard *et al.,* 2004; Darias *et al.,* 2015). Currently, there is a dearth of reported studies to unmask natural products of non-protein substitutes that could maximize the use of costly protein and increase feed efficiency.

Sweet potato (*Ipomoea batatas)* is a dicotyledonous plant, which belongs to the morning-glory or convolvulaceae family. Locally, it is known as *camote.* It is an herbaceous creeping plant with smooth, lightly moderate green leaves sometimes with a considerable amount of purple pigmentation along its veins (Arifina *et al.,* 2020). Sweet potato leaves contain 4.90% crude fat; 24.85% crude protein; 51.95% carbohydrate; 7.20% crude

* Corresponding author. e-mail: debbaldo@parsu.edu.ph.

fiber; and the caloric value is estimated at 351.30 ME kcal/g (Antia *et al.,* 2006). However, the plant's potential as economic source is unrecognized due to its concept as food to the common population and traditional feed to domestic animals (Oyenuga, 1968). Sweet potato is dominantly found in the tropics like the Philippines. The abundance of this plant source provide justification to its possible use as meal ingredient in aquaculture. Therefore, the present study assessed the effects of *I. batatas* feed supplementation on blood profile and tissue histoarchitecture of ammonia-exposed *O. niloticus*. Specifically, it aims to determine the weekly hematological response in red blood cells (RBCs), white blood cells (WBCs), and blood platelets components, besides examine the histological alterations in the liver, kidney, and gills of experimental fish.

2. Materials and Methods

2.1. Plant preparation

Sweet potato red shoots were purchased at Goa public markets in Camarines Sur, Philippines with a total number of 305 bundles. Samples were stationed at the Science Laboratory of Partido State University Natural Science Laboratory. Leaves were washed and air-dried for 72 hours. Thereafter, the leaves were reduced to fine powder using mechanical blender. Powdered sweet potato leaves are stored for extraction procedures.

2.2. Preparation of hot water extract

Hot water extraction was performed according to Kim *et al.* (2011). Pulverized sweet potato leaves at 50 g were added to 500 mL of pure distilled water. The solution was boiled in a hot plate for 3 hours at temperature not exceeding 100°C. The suspension was passed through a nylon mesh. The filtered hot water extract was frozen and maintained in a storage until the feed formulation.

2.3. Preparation of crude extract

Pulverized sweet potato leaves at 500 g were soaked in 5 L of analytical grade ethanol for 72 hours. Thereafter, the samples were filtered using Whatman paper no. 42. The filtrate was collected and concentrated using rotary evaporator (IKA-100) under reduced pressure at 45°C in 100 rpm. The extract was refrigerated at 4°C until the feed formulation.

2.4. Fish culture and feeding experiment

A total of 240 male and female *O. niloticus* with mean weight 14.91 ± 1.59g were collected at PSU-Sagnay Multi-species Hatchery and were acclimated for 1 week in 1 m^3 concrete tanks. Experimental fish were randomly distributed in four treatments with three replications, each replicate was assigned with 20 fish. During the acclimatization period, fish fed the basal diet. The water was aerated with electric pumps and was renewed once every three days. Water quality parameters such as dissolved oxygen (DO), and temperature were monitored regularly and maintained using DO-meter and tank thermometer, respectively.

2.4.1. Diets and experimental design

Fish in the control group (T1) fed a practical diet described by FAO (2021) with 0% of sweet potato leaves. Other groups received the practical diet (95%), supplemented with 5% of either sweet potato leaves powder (T2), hot-water extract (T3) and crude extract (T4). Diet ingredients were grounded, mixed, and pelleted using animal feed pellet machine. The respective diets were provided twice on a daily basis (8:00 AM and 4:00 PM) and consisted with 5% of the live body weight of the fish.

2.5. Blood collection and hematological analysis

Twelve fish were randomly collected from each treatment. Blood samples at 0.5 ml per fish were collected at weeks 2, 4, 6, 8, 10 and 12, from the caudal peduncle region using 1 cc (25 g × 5/8) syringe rinsed with ethylenediaminetetraacetic acid (EDTA) as anti-coagulant. The samples were analyzed using Rayto Auto Hematological Analyzer (RT-7600) to determine various hematological parameters such as red blood cells (RBCs), hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cells (WBCs), neutrophils, lymphocytes, monocytes, eosinophil, platelet, mean platelet volume (MPV) and platelet distribution width (PDW).

2.6. Ammonia toxicity experiment

A range finding test was conducted to determine the sub-lethal dose of ammonia following the methods described by El-Shafai *et al.* (2004), with few modifications. After the 12-week feeding experiment, the fish were exposed to 6 mg L^{-1} concentration of ammonia (Loba Chemie, India) continuously supplied with aeration. The fish were observed in a 24-hour duration (Küçük, 2014). Fish demonstrating morbidities and mortalities were sacrificed. Ice was added incrementally, and was used as a non-chemical anesthesia (Coyle *et al.,* 2004). Kidney, liver and gills were harvested for histopathological examination.

2.7. Histological analysis

A total of four random fish from each group were sacrificed. In brief, fragments from target organs such as kidney, liver and gills were harvested. The samples were then fixed in 95% formaldehyde solution. Subsequently, samples were dehydrated, cleared using xylene and embedded in paraffin. Segments were cut at 3 µm via a microtome. Samples were stained with hematoxylin and eosin (H&E) for histopathological analysis.

2.8. Statistical analysis

Data are shown as mean ± standard error of the means (SEM). One-way analyses of variance (ANOVA) was performed using IBM SPSS Statistics 26.0 software. Differences between treatment means were compared by Tukey's test. The significant differences between mean were statistically considered at $P<0.05$.

3. Results

In this study, the effect of sweet potato incorporation in feeding diets on blood profile and tissue histomorphology of *O. niloticus* was evaluated. The latter effect was achieved by ammonia exposure within 24 hours. Data on the changes in hematological parameters are presented separately in Tables 1, 2, and 3. While, the histological

characterization of tissues from liver, kidney and gills are shown in Figures 1, 2 and 3, respectively.

3.1. Changes in RBCs, WBCs and Platelet count

In Table 1, the data suggest no significant difference in hematocrit throughout the feeding duration. At week 2, significant (P<0.05) difference was observed in the three formulated diets in terms of MCV. At week 8, RBCs recorded significant difference among treatments, where T4 and T2 displayed the lowest and highest count, respectively. This record is inverse with those of MCHC in the same week. At week 12, T2 significantly showed a high level of hemoglobin (P<0.05).

Table 1. Record of erythrocyal components of *Oreochromis niloticus* fed *Ipomoea batatas* for the 12-week feeding experiment

	Treatment	Culture period (week)					
		2	4	6	8	10	12
Red blood cells (10^6 mL^{-1})	T1	1.81±0.08	1.97±0.05	2.05±0.10	2.00±0.06^{ab}	2.00±0.12	1.73±0.09
	T2	1.78±0.14	2.00±0.08	2.11±0.14	2.08±0.06^{b}	1.95±0.17	1.88±0.13
	T3	1.93±0.07	1.93±0.13	1.96±0.12	1.84±0.08^{ab}	1.99±0.15	1.94±0.13
	T4	1.67±0.11	1.59±0.23	1.85±0.11	1.78±0.08^{a}	1.73±0.14	1.85±0.12
Hematocrit (%)	T1	24.4±1.32	23.68±0.83	28.38±1.09	31.07±1.41	28.97±1.09	26.23±1.74
	T2	28.22±2.78	26.97±0.93	29.75±1.40	31.47±1.04	26.60±1.99	30.37±1.51
	T3	28.62±0.66	26.60±0.99	27.77±1.61	28.13±0.59	30.15±1.27	28.47±1.34
	T4	26.80±1.54	22.07±3.10	27.75±1.04	28.05±1.27	25.90±2.16	26.25±1.16
Hemoglobin (g dL^{-1})	T1	105.33±6.87	104.33±3.19	116.17±5.18	124.17±2.37	114.33±5.38	113.67±5.42^{a}
	T2	115.33±7.63	117.67±3.74	112.83±5.11	121.50±8.01	119.00±5.33	136.00±4.22^{b}
	T3	118.00±1.62	119.33±3.45	105.17±5.51	123.00±2.81	132.17±2.60	129.50±2.27^{ab}
	T4	109.00±5.19	100.00±13.5	114.33±6.07	126.17±3.44	107.17±9.36	119.67±4.07^{a}
Mean Corpuscular Volume (fl)	T1	134.95±5.99^{a}	119.93±3.03	140.17±8.13	155.38±3.14	146.33±4.19	151.05±4.16
	T2	157.63±3.89^{b}	119.10±20.14	142.33±5.10	151.02±2.70	138.82±6.60	153.28±7.88
	T3	149.23±3.53^{b}	139.80±6.40	142.07±3.54	153.82±4.80	154.80±8.15	144.57±4.60
	T4	161.10±3.08^{b}	137.25±5.13	142.88±4.29	157.88±1.54	149.55±4.11	147.20±6.83
Mean Corpuscular Hemoglobin Concentration (g dL^{-1})	T1	431.67±10.44	441.33±7.32	401.67±10.72	412.50±8.63^{ab}	393.50±5.92	436.33±17.98
	T2	476.50±48.73	438.0±6.11	380.83±11.26	385.0±18.87^{a}	426.83±8.83	453.0±14.91
	T3	412.50±4.65	451.83±16.25	380.83±10.60	437.83±13.26^{ab}	441.83±12.96	450.50±13.61
	T4	413.67±22.32	458.17±21.61	411.17±7.72	451.67±13.26^{b}	416.50±17.50	438.0±.28

Values are presented as mean±SEM; n=12
Different letters indicate significant difference, P<0.05

For leukocytal records, no significant difference was observed in the white blood cells and monocytes (Table 2). At week 6, leukocytes level from the three formulated diets of *I. batatas* revealed significant increase. In the same week, neutrophil count presented the opposite trend with T1 showing significant increase (T2,>T4>T3). At week 8, T1 and T3 showed significant elevations in lymphocytes. Also, T2 group showed significant increase in eosinophils levels (P<0.05).

Table 2. Record of leukocytal components of *Oreochromis niloticus* fed *Ipomoea batatas* for the 12-week feeding experiment

	Treatment	Culture period (Week)					
		2	4	6	8	10	12
White blood cells (10^3 mL^{-1})	T1	60.18±3.82	65.91±6.87	80.11±6.47	76.77±5.80	63.26±4.58	76.76±5.83
	T2	70.39±7.09	63.91±2.54	85.67±3.82	84.42±3.26	66.63±7.79	78.36±5.06
	T3	62.40±1.99	68.06±2.89	86.27±5.71	92.44±7.83	75.09±3.11	85.60±4.50
	T4	61.55±4.85	59.81±10.77	88.29±4.51	70.14±2.84	59.50±8.77	68.85±3.93
Neutrophils (10^3 mL^{-1})	T1	19.14±3.00	11.85±3.47	15.96±3.43^{b}	12.09±7.66	5.14±2.89	7.62±3.86
	T2	16.80±2.49	16.10±3.26	6.77±2.67^{ab}	14.84±3.72	9.13±4.72	13.69±8.01
	T3	12.56±3.24	15.54±2.52	2.37±0.99^{a}	13.29±5.08	7.58±2.34	14.90±3.45
	T4	17.15±4.42	12.30±4.31	6.60±2.06^{ab}	25.57±3.74	6.60±2.19	8.17±2.96
Lymphocytes (10^3 mL^{-1})	T1	16.67±2.66	35.78±14.42	38.47±6.93^{a}	50.89±12.01^{ab}	54.64±7.26	43.20±9.41
	T2	20.68±5.47	19.63±3.35	70.06±5.78^{b}	41.31±10.7^{b}	51.66±8/86	48.95±7.60
	T3	28.20±8.62	18.34±2.29	74.60±6.91^{b}	64.82±12.74^{ab}	61.43±5.89	60.06±4.54
	T4	20.86±8.31	29.06±11.43	66.81±6.49^{b}	8.66±1.37^{b}	43.70±7.41	54.88±2.26
Monocytes (10^3 mL^{-1})	T1	22.29±2.54	17.06±6.05	21.10±4.18	11.93±6.84	1.95±1.66	11.10±4.52
	T2	30.54±6.12	26.59±1.44	6.22±5.48	25.46±8.19	6.38±3.65	7.95±3.47
	T3	22.16±6.39	32.32±4.42	5.64±3.93	12.90±6.73	3.40±2.59	13.44±4.04
	T4	22.91±5.46	16.69±5.14	11.78±4.89	32.80±1.61	6.59±2.25	7.19±4.43
Eosinophils (10^3 mL^{-1})	T1	1.99±0.48	1.23±0.16	4.55±1.62	1.84±0.52^{ab}	1.52±0.31	1.06±0.20
	T2	2.39±1.14	1.60±0.25	2.58±1.21	2.82±0.34^{b}	2.78±1.06	2.27±0.56
	T3	1.26±0.27	1.84±0.41	0.82±0.24	1.39±0.25^{a}	2.66±0.51	2.16±0.69
	T4	0.61±0.13	1.77±0.42	3.11±0.94	0.89±0.18^{a}	2.56±0.93	2.20±0.21

Values are presented as mean±SEM; n=12
Different letters indicate significant difference, P<0.05

For platelet monitoring (Table 3), no significant difference was observed from weeks 2 to 12 in the MPV. Platelet and PDW were shown to present early changes in the three *I. batatas* supplemented feeds as compared to those fed the practical diet (T1) at week 2 (P<0.05). At week 8, platelet level significantly accelerated in T3 (P<0.05). At week 12, T1 and T2 showed significant increase in the PDW.

Table 3. Record of thrombocytal components of *Oreochromis niloticus* fed *Ipomoea batatas* for the 12-week feeding experiment

	Treatment	Culture period (week)					
		2	4	6	8	10	12
Platelet (10^6 mL^{-1})	T1	9.00±0.94^a	12.17±3.03	12.17±1.44	42.83±10.59^a	20.17±3.23	58.83±28.68
	T2	16.17±2.13^b	12.67±1.48	18.83±4.09	118.17±10.59^b	35.67±14.85	94.83±39.87
	T3	16.17±2.13^b	15.67±3.33	36.00±16.14	44.00±9.84^a	22.00±3.23	21.83±6.07
	T4	14.33±0.87^b	14.50±1.88	19.33±3.08	27.83±4.04^a	17.00±3.55	85.33±40.72
Mean platelet volume (fl)	T1	7.35±0.37	6.82±0.37	9.17±0.54	9.55±0.46	10.10±0.44	10.63±1.29
	T2	8.43±0.38	8.33±0.61	9.18±0.39	11.30±0.98	10.63±1.01	11.72±1.4
	T3	8.30±0.35	8.78±0.44	8.98±0.79	9.32±0.55	10.67±0.29	9.55±0.4
	T4	8.43±0.27	7.90±0.75	9.20±0.58	10.18±0.29	9.30±0.62	10.38±1.46
Platelet distribution width (fl)	T1	16.22±0.46^a	16.22±0.49	18.10±0.36	17.60±0.46	17.37±0.54	16.82±0.60^{ab}
	T2	17.83±0.30^b	17.48±0.45	17.92±0.31	16.85±1.22	18.15±0.32	16.20±0.58^{ab}
	T3	18.02±0.32^b	17.62±0.37	17.78±0.67	18.52±0.43	17.60±0.50	17.58±0.58^b
	T4	17.68±0.35^b	17.33±0.49	18.25±0.36	18.58±0.16	17.40±0.47	15.13±0.49^a

Values are presented as mean±SEM; n=12
Different letters indicate significant difference, P<0.05

3.2. Histopathological alterations of organs

3.2.1. The gills tissues

Gills tissues (Figure 1 A-D) show no notable amelioration in any of the experimental groups. All samples demonstrated destruction in the histomorphology of gills as observed by the infiltration of inflammatory cells, predominantly lymphocytes in the primary and secondary lamella. However, more tissue anomalies are exhibited in the T2 and T3 such as hyperplasia with foci of necrosis. Gills from T4 displayed foci of congestion in primary and secondary lamella, primary lamellar epithelial lifting, fusion and clubbing of lamellar tips.

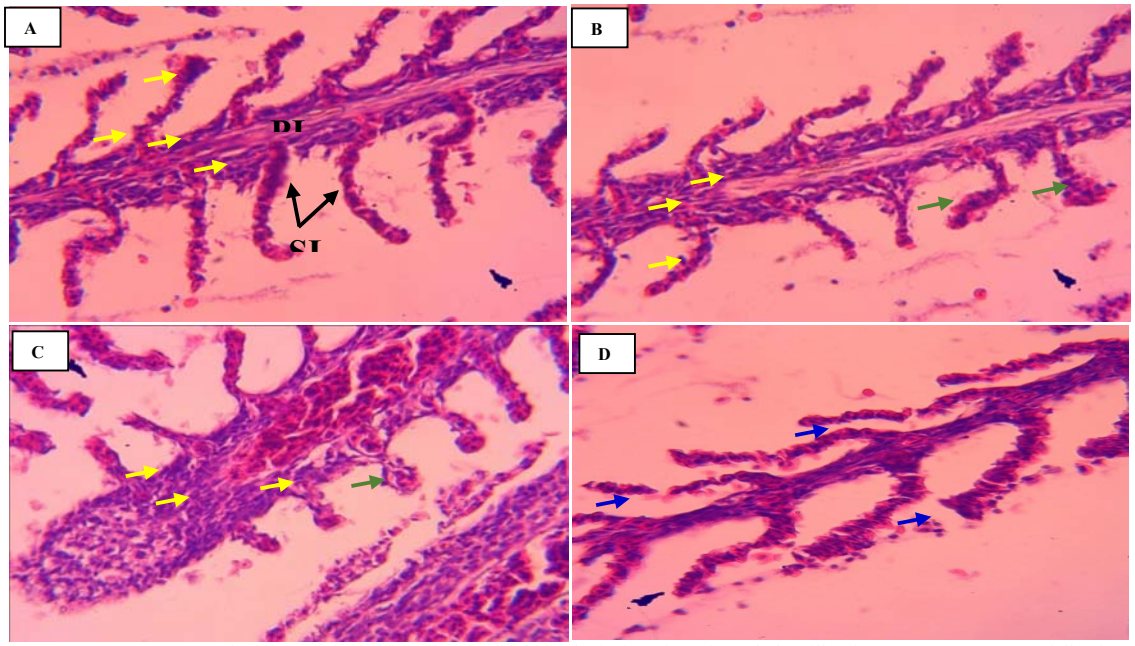

Figure 1 (A-D). Representative photomicrographs of longitudinal histological sections through the gills of *Oreochromis niloticus* following 24-hr exposure to ammonia from experimental fish fed the practical diet (A), sweet potato powder (B), sweet potato hot water extract (C), and sweet potato crude extract (D), showing primary lamellar epithelium (PLE), secondary lamellae (SL), widespread infiltration of inflammatory cells in the primary and secondary lamella (yellow arrow), hyperplasia with foci of necrosis (green arrow), epithelial lifting and fusion of lamellar tips (blue arrow). (H&E, 400×).

3.2.2. The kidney tissues

The histological alterations in kidney tissues of all treatments are briefly illustrated in Figure 2 (A-D). The feeding supplementation demonstrated no significant effect in the histopathology of kidney tissues except for the decrease of melanomacrophages (MMC), compared to control group. T2 and T3 illustrated prominent necrosis of tubular epithelium and hepatic cord disorder accompanying with hepatocyte hypertrophy and cloudy swelling. Also spotted are foci of mineralization in some tubules. T4 group showed aggregates of lymphocytes around blood vessels.

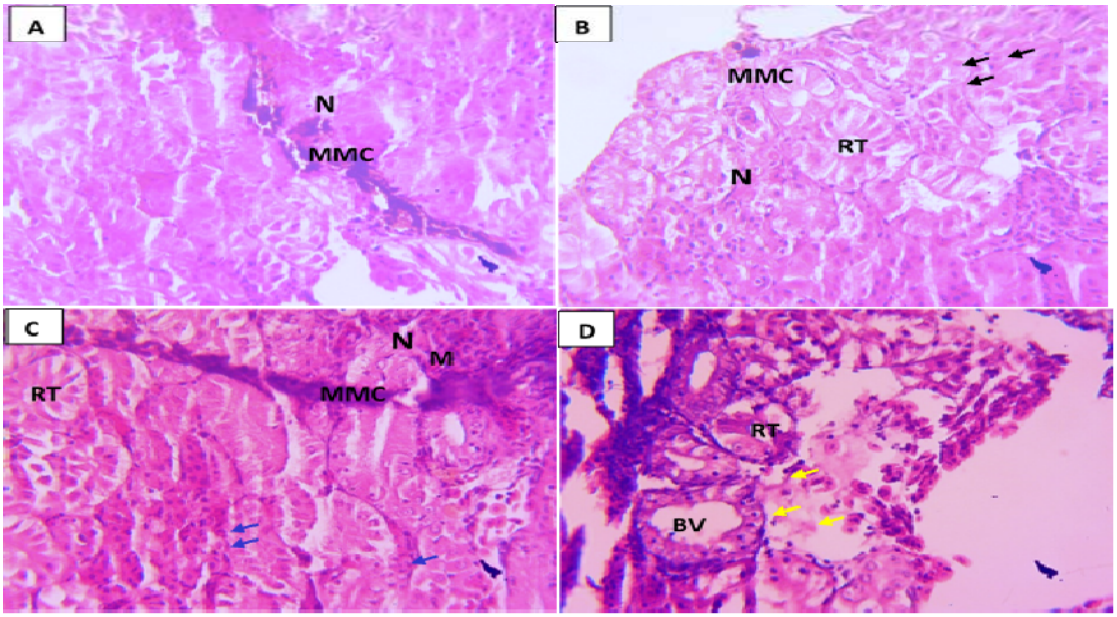

Figure 2 (A-D). **Representative photomicrographs of kidney tissues of *Oreochromis niloticus* following 24-hr exposure to ammonia** from experimental fish fed the practical diet (A), sweet potato powder (B), sweet potato hot water extract (C), and sweet potato crude extract (D), showing moderate depletion of hemopoietic tissues in the interstitium surrounding the tubules (black arrow), increased numbers of melanomacrophages (MMC) within the interstitium, prominent necrosis of tubular epithelium (N), hepatic cord disorder accompanying with hepatocyte hypertrophy and cloudy swelling (blue arrow), foci of mineralization in some tubules (M), few aggregates of lymphocytes (yellow arrow) around blood vessels (BV), renal tubules (RT). (H&E, 400×).

3.2.3. The liver tissues

Surprisingly, histopathology of liver (Figure 3 A-D) showed normalized liver in the control group receiving the practical diet for 12-week of the feeding trial. There were no pathological abnormalities, with hepatocytes presenting a homogenous cytoplasm, and a large central or sub-central spherical nucleus. In both of T2 and T3, moderately damaged liver was exposed. The histopathological changes found in the liver of examined fish included irregular arrangements of hepatocytes, vacuolation, and erythrocyte infiltration in the hepatic tissues. Comparable to the control group, T4 showed nearly normalized liver histology characterized by prominent normal size hepatocytes with central and sub-central spherical nucleus.

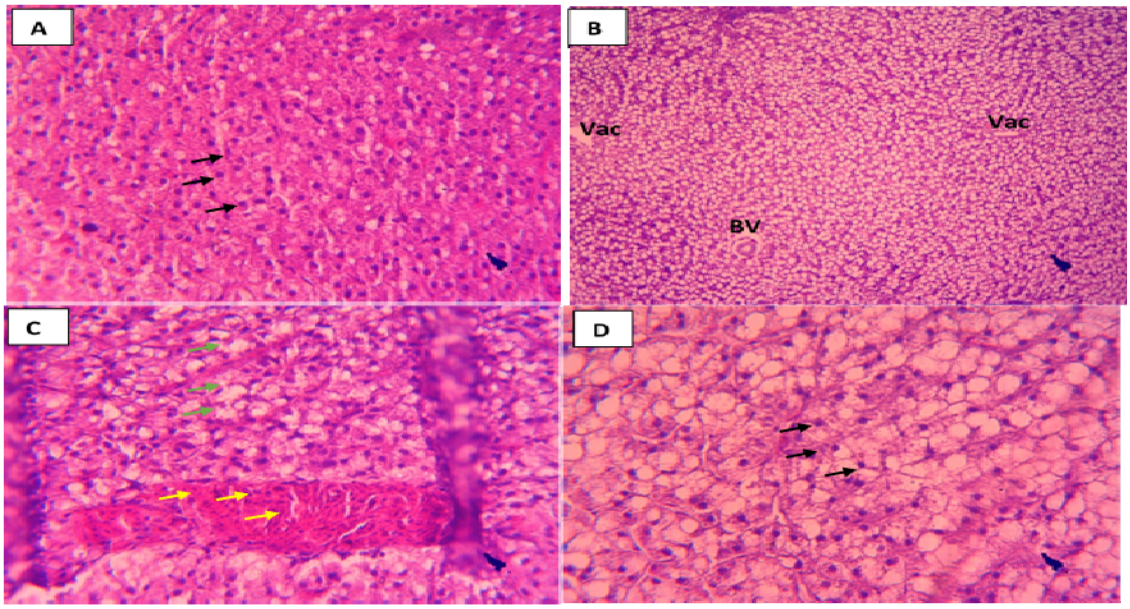

Figure 3 (A-D). Representative photomicrographs of liver tissues of *Oreochromis niloticus* following 24-hr exposure to ammonia, from experimental fish fed the practical diet (A), sweet potato powder (B), sweet potato hot water extract (C), and sweet potato crude extract (D), showing normal hepatocytes (black arrow), mild loss of cytoplasmic vacuolations (vac), blood vessels containing proteinaceous fluid (BV), erythrocyte infiltration into blood sinusoids (yellow arrow) and increased vacuolation of hepatocytes (green arrow). (H&E, 400×)

4. Discussion

Hematological parameters are important indicators of the fish's physiological and health status. According to Sahan *et al.* (2016) WBCs, RBCs, hematocrit and hemoglobin monitoring is instrumental in maintaining the stock in fish farms. More authors prove that botanical immunostimulants have bioactive substances that can cause increase in blood cell counts, thereby triggering immunity and enhance natural defense in fish species (Ajeel and Al-Faragi, 2013; Haghighi and Rohani, 2013; Talpur *et al.,* 2013).

In this study, significant increase has been reported in the MCV, and PDW at week 2, as all formulated diets exhibited significant increase compared to the practical diet. This trend was further shown at week 6 in the levels of lymphocytes. These elevations determined increase in the natural defense cells number. Neutrophils significantly increased in the practical diet–fed group. These results are indicative of early enhancement of immune cells. Indistinct pattern on week 8 has been recorded as blood components in the group receiving T4 significantly decrease (lymphocytes, eosinophils, RBCs and platelet) compared to other groups. The increase in RBCs can be a result of an improved oxygen supply, mirroring a more efficient blood circulation supply of the organism (Ruiz *et al.,* 2020).

Formulated feeds from sweet potato effected to no significant difference in terms of WBCs at any monitoring week. This result is in corroboration with the findings reported by Yones *et al.* (2019), where increasing levels of carbohydrates from wheat bran, corn and sorghum was used as alternative source of carbohydrates to tilapia culture. Lowest WBCs can be observed in T4 or crude extract supplemented feed group, indicative of immunosuppression in the experimental fish.

In the present study, histological alterations in three important organs was studied, gills for examining respiration in respect to exposure to toxins, liver for metabolism of toxicants, and kidney for its involvement in the elimination process. After the 24-hour exposure to 6 mg ammonia L^{-1}, samples from all experimental set-ups revealed notable anomalies in the gill histoarchitecture. According to the study of Le Ruyet *et al.* (1998) ammonia enters the fish 15 minutes following exposure. Initial effects of such contaminants are evidenced in the cellular and sub-cellular levels starting from the first hour of exposure. There are also reports mentioning that chronic exposure to nitrate results to change in the swimming patterns and more importantly to the health of the fish (Davidson *et al.,* 2014). In this study, histological effects in gill tissues agrees to findings of previous authors (Yones *et al.,* 2019; Ruiz *et al.,* 2020), where hyperplasia of the lamella, widespread infiltration of inflammatory cells and epithelial lifting and fusion of lamellar tips were observed after exposure to ammonia. No evidence of gills histology normalization in any of the treatment were demonstrated by all the formulated feed diets, suggesting the inability of the feed components to secrete and continuously replace their mucus layer as a biological barrier (Yoon *et al.,* 2015). For kidney tissue examination, evident decrease in MMCs was observed in all fish receiving sweet potato-incorporated diets. MMCs are aggregates of highly pigmented phagocytes found primarily in the head kidney and spleen, and occasionally on livers (Steinel and Bolnick, 2017). The decline in the numbers of MMCs could be related to an adaption of fish to the recirculation system, allowing a decline of natural stress conditions. MMCs are developed in cases involving chronic inflammatory lesions, in the destruction, detoxification and recycling of endogenous and exogenous materials. Also, it could be indicative of environmental stress and mirrors activity immune response to microbial antigens (Agius and Roberts, 2003). On the other hand, the liver is a vital organ involved in the physiological processes of metabolism, and excretion. Thus, it plays a pivotal role in the removal of toxic substances from the bloodstream and final excretion. In the current study, histoarchitecture of the fish liver tissue was damaged in T2 and T3 groups, as revealed by the increase in hepatocellular basophilia, moderate loss of cytoplasmic vacuolations and few blood vessels containing proteinaceous fluid. Vacuolation may indicate stored energy in the form of glycogen or lipid, and may also express a degenerative change in cellular organelles like the endoplasmic reticulum and Golgi apparatus (Braunbeck, 1998). Furthermore, observable vacuolations may be reflective of an accumulation of free fluid in the cytoplasm (Stehr *et al.,* 1998). Fluid build-up causes distention of the liver cell cytoplasm resulting to ballooning degeneration or cloudy swelling, which was evident in the T3 group. On the other hand, crude extract of sweet potato when added as feed ingredient was able to display normalized liver histoarchitecture. These formulated feeds were mentioned to contain high amounts of total carbohydrates with the crude extract presenting the highest value of 34.94% compared to 34.20% and 34.63% of hot water extract and sweet potato powder, respectively (Pallaya-Baleta *et al.,* 2021). Thus, the possible mechanism that may have occurred as result of normalized liver tissue in T4-fed group is the optimal amounts of carbohydrates present. Metabolism of such may have improved the liver histology after the toxicant contamination. The consequence of this study agrees with the findings communicated by Ishak *et al.* (2016) who concluded that fish fed carbohydrates at 30% show minor histological alterations with increased occurrence of lipid vacuolization in hepatocytes and reduced expression of nucleus that would indicate disintegrated liver cells.

The current findings are early confirmed with reports on high levels of protein and non-protein energy source of sweet potato (An, 2004; Ekenyem and Madubuike, 2006). Furthermore, Ishida *et al.* (2000) and Ekenyem and Madubuike (2006) indicated that the leaf of sweet potato has high protein content (26% to 35%) and ideal minerals quantities of vitamins such as A, B2, C, and E. The present study further supports the early claim of Oyenuga (1968) on the rich nutrient qualities of sweet potato leaves and recommends the use of sweet potato leaves as source of feed supplement in aquaculture.

5. Conclusion

From the obtained findings, it could be concluded that supplementation of the crude extracts of sweet potato leaves in practical diets of *Oreochromis niloticus* can significantly improve hematological profile and eventually

the fish immune response. Similarly, the incorporation of the crude extracts displayed activity in the enhancement of liver and kidney histopathology against ammonia as an environmental toxicant. Finally, sweet potato is warranted as preferable feed additive supplement, capitalizing on its non-protein source such as carbohydrates as bioactive component.

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