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Original Article

Stress response of tilapia, *Oreochromis niloticus*, when fed diets supplemented with sweet potato (*I. Batatas*) shoots and exposed to ammonia♣

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Abstract

The present study investigated the hematology, survival and behavior of *Oreochromis niloticus* that received formulated diets (FD) with *Ipomoea batatas* shoots (IbS) as powder or extracts, when exposed to sub-lethal dose of ammonia. The study had four experimental treatments: a control treatment that received formulated diets (FD) only and three treatments that received FD with 5% *I. batatas* shoots powder (FD+PIbS), hot-water extract (FD+HWEIbS), and crude ethanol extracts (FD+CEEIbS). After 12 weeks of feeding, the fish were exposed for 21 d to a sub-lethal dose of ammonium chloride causing the stress response. During the exposure an increase in the leukocyte indices (white blood cells, WBC; neutrophils, NEU; lymphocytes, LYM; monocytes, MON) was observed in the fish that received FD only while the fish that received FD with *I. batatas* remained comparable. In contrast, the erythrocyte indices (red blood cells, RBC; hematocrit, HCT; hemoglobin, HGB) of the fish that received *I. batatas* were elevated and significantly different ($P<0.05$) from the control group. The platelet (PLT) count of the fish that got FD+HWEIbS was significantly higher than in the other treatments. In terms of survival rate, the fish that received FD+HWEIbS was top ranked. The swimming activity of the fish was reduced during the exposure, as a change in behavior. In conclusion, nutritional supplementation of *I. batatas*, particularly as hot-water extract, enhanced tilapia's resistance to physiological stress such as from ammonia exposure, as evidenced by its haematological profile and high survival.

Keywords: sweet potato powder and extract, ammonia toxicity, tilapia hematology

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1. Introduction

The Nile tilapia (*Oreochromis niloticus*) is a popular aquaculture fish because of its strong tolerance to a broad range of environmental conditions, high growth performance, and high market value (El-Sayed, 2015). Because of these desirable attributes, the culturing of tilapia has intensified in the past decades. This development includes increased stocking density, environmental and cultural condition management (water quality, feeds, and feeding management), and more. However, these practices may result in also negative impacts, such as overcrowding, pollution, and stress; and may eventually affect the health status of the fish.

Ammonia, which is produced by unconsumed fish feed and/or feces, is a prevalent contaminant in intensive culture systems (Lin and Chen, 2003). There are two forms of ammonia in water, the un-ionized (NH3) and ionized forms (NH4+). NH3 is generally linked with ammonia toxicity because it can easily diffuse across gill tissues (Ebeling *et al.*, 2006).

According to a study by Chakraborty and Hancz (2011), plant extracts stimulate development and improve immunostimulation in fish and shrimp aquaculture. Furthermore, plant extracts improve digestibility, leading to a higher feed conversion rate (Citarasu, 2010; Nya, & Austin, 2009; Talpur *et al.*, 2013). Immunostimulants and other natural products help fish maintain their health and improve their immune response (Yin *et al.*, 2008).

Sweet potato (*Ipomoea batatas*) is a traditional tropical crop, especially in China and Southeast Asia, where it is widely cultivated (Statistical Yearbook, 2002). According to Ekenyem and Madubuike (2006), *I. batatas* leaves have a high protein content (26-33%) and are a good source of antioxidants, B vitamins, minerals, and dietary fiber, which may help the tilapia to withstand ammonia toxicity. The present study aimed to determine the behavior, survival and haematological profile of tilapia fed with diets containing *I. batatas* powder or extracts, when exposed to a sub-lethal dose of ammonia. Tilapia culture encourages health management against environmental problems. This may include such improvements of aquaculture operations that lead to reduced costs and better stability of the production system, and the current study offers a potential approach to make the system more robust.

2. Materials and Method

2.1 Preparation of *I. batatas* extract and powder.

Fresh *I. batatas* shoots were purchased from the public market of Goa, Camarines Sur. The shoots were air-dried for 14 days and pulverized using a mechanical blender. The hot-water extract of *I. batatas* (HWEIbS) was prepared following the method described by Fujiki *et al.* (1992), Hou and Chen (2005) and Baleta *et al.* (2013). The powdered *I. batatas* (PIbS) was mixed with distilled water in a 1:10 ratio. The mixture was boiled for 3 h in a water bath set-up. After boiling, the suspension was passed through a nylon mesh for filtration. The filtrate extract was used for HWEIbS diets. CEEIbS was prepared by soaking PIbS in 95 % ethanol at a ratio of 1:10 for 72 hr. It was then filtered using Whatman no. 42 filter paper and dried using rotary evaporation (IKA-100) under reduced conditions (40 oC). The PIbS, HWEIbS and CEEIbS were added to formulated diets at 5% inclusion level.

2.2 Formulation of experimental diets

Each ingredient and its percentage inclusion per kilogram of diet is presented in Table 1. To ensure homogeneity, the ingredients were finely ground using a miller and mixed by hand. After mixing, the dough was formed and steamed for 10 minutes. The steamed dough was pelleted using a mechanical pelletizer (3 mm) and air-dried at room temperature for two days, then stored in a clean container until use.

2.3 Experimental design and treatments

Experimental fish: The study used 240 tilapia (*O. niloticus*) fingerlings with a mean weight of 11.64±1.85 g. The fish were reared in 1 m x 1 m x 1 m concrete tanks at Partido State University Sagñay Campus Multi-species hatchery Laboratory. The fish were given a 15 d acclimation period before actual feeding of the Formulated diet (FD). They were fed on a commercial diet during the acclimation period.

Table 1. Percentage inclusion levels by weight of the ingredients in experimental diets

Feed ingredient	Experimental groups			
	FD (%)	FD+PIbS (%)	FD+HWEIbS (%)	FD+CEEIbS (%)
Fish Meal (local)	25	28	28	28
Corn Meal	12	10	10	10
Soy bean meal	28	27	27	27
Rice bran	21	16	16	16
Corn Oil	5	5	5	5
Vit. And Minerals	4	4	4	4
Corn Starch	5	5	5	5
Powered *I. batatas*	-	5	-	-
Hot-water extract of *I. batatas*	-	-	5	-
Crude ethanolic extract of I. batatas	-	-	-	5

Note: FD-Formulated Diet, FD+PIbS- Formulated Diet with Powdered Ipomoea batatas Shoots, FD+HWEIbS- Formulated Diet with Hot-Water Extract of Ipomoea batatas Shoots, FD+CEEIbS- Formulated Diet with Crude Ethanolic Extract of Ipomoea batatas Shoots

Feeding experiment: After the acclimation period, the fish were randomly divided into four groups (30 fish per group). Each group consisted of three replicates (10 each). The treatment groups were: a control group that received formulated diet (FD), and three groups that received formulated diets with powdered *I. batatas* shoots (FD+PIbS), hot-water extract of *I. batatas* shoots (FD+HWEIbS), and crude ethanol extract of *I. batatas* shoots (FD+CEEIbS). The diet was given to the fish twice daily at 8 am and 3 pm for 12 wks.

Range finding test: A range finding test in static laboratory conditions was conducted following the protocols of APHA (1975), EPA (1998) and ISO (1996) to determine the median lethal concentration (LC50) of ammonium chloride to be used in the stress response experiment. Briefly, ten fish were stocked in each 100 liter capacity aquarium with pre-aerated water mixed with commercially-available ammonium chloride solution (1, 3, 6, 9 and 12 mg per liter). Mortality and physiological conditions (behavioral response) of the fish were recorded every hour for the first 24 hr and then every 24 hr until 96 hr.

2.4 Stress response experiment

The stress response experiment was carried out after 12 weeks of feeding. The fish were exposed to a sub-lethal dose of commercially available ammonium chloride solution. The ammonium chloride was purchased from a local drug store and mixed with pre-aerated water using standard methods. The fish was exposed to ammonia for 21 days. Fish were fed on a commercial diet (32% crude Protein, 3% crude fat and 5% crude fiber) to satiation twice daily at 8 am and 3 pm. Settled fish wastes were siphoned every day and water was replaced with maintained ammonia concentration.

2.5 Blood collection and hematological analysis

Six fish were randomly collected from each treatment after 1hr at day 1, and at 3, 5, 7, 14, and 21 d of exposure to ammonium chloride. Blood (0.5 cc) was withdrawn intraperitoneally near the caudal peduncle region of the fish using a 25 g x 5/8 syringe rinsed with EDTA as anti-coagulant. Each collected blood sample was transferred in a 0.5 ml vacoutainer tube with EDTA and was analyzed using Rayto Auto Hematological Analyzer (RT-7600).

2.6 Statistical analysis

The value of each experimental parameter is expressed as mean ± SE. The treatment means were compared using the Student-Neuman Keul's test (IBM SPSS version 26 for Windows 10). A probability level of 0.05 was used to call the significances of differences between treatments.

3. Results and Discussion

3.1 Haematological profile and survival

Haematology and stress response of the fish are the important metrics to determine the health and to assess the fish feed effects. After the range finding test for LC50¬¬ the final concentration computed was 6 mg per liter, so that after 12 weeks of feeding, the fish were exposed for 21 d to 6 mg per liter of ammonium chloride. After the exposure an increase in the leukocyte indices (white blood cells, WBC; neutrophils, NEU; lymphocytes, LYM; monocytes, MON) was observed in the fish that received FD only (Table 2) agreeing with the study of Das *et al.* (2004) on the WBC of Mrigal in response to nitrite and of red tilapia to 3 mg per liter NH4Cl for 3 d (Bonnie, & Liu, 2004). According to Das *et al.* (2004) the increase in WBCs as a result of exposure to stresses is involved in the regulation of immunological function of the fish.

The hemoglobin, hematocrit, and mean corpuscular volume of fish fed with FD+PIbS was significantly (P<0.05) higher compared with the fish fed with FD+HWEIbS, FD+CEEIbS or FD only. The reduction of the hemoglobin in the present work has similar trend as in the study of El-Sherif and El-Feky (2008) wherein Tilapia fish were exposed to 0.15 mg per liter NH3 for 60 days. The decrease in hemoglobin in the case of common carp (*C. carpio*) exposed to ammonia is caused by an increase in the oxygen intake and elevation in methemoglobin associated with gill damage (Tilak *et al.*, 2007).

There were no significant differences in MCH by diet. However, the percentage of MCHC exhibited significant (P < 0.05) decreases in fish fed with FD+HWEIbS compared with the other treatments. The increases in HGB, HCT, MCV, MCHC, neutrophils, and lymphocytes of the fish fed with *I. batatas* in the diet indicate the immunostimulatory effects and anti-infection properties of the plant, and may be related to the

Table 2. WBC and Leukocyte indices of tilapia after 21 d of exposure to ammonia

Treatment	Leukocyte indices				
	White blood cell (10^6/ml)	Neutrophil (10^6/ml)	Lymphocyte (10^6/ml)	Monocyte (10^6/ml)	Eosinophil (10^6/ml)
FD	76.90±2.02[a]	11.16±2.94[a]	71.70±1.45[a]	15.63±3.51[a]	0.75±0.39[a]
FD+PIbS	62.33±1.46[b]	5.20±1.49[a]	58.17±1.21[b]	6.18±2.72[a]	1.83±0.60[a]
FD+HWEIbS	57.88±6.01[b]	4.57±1.31[a]	55.20±5.61[b]	6.24±1.55[a]	0.35±0.13[a]
FD+CEEIbS	67.55±3.57[ab]	7.07±1.59[a]	63.62±3.09[ab]	11.55±2.34[a]	0.77±0.32[a]

Note: Means with different letters as superscripts are significantly different from each other (P< 0.05). FD-Formulated Diet, FD+PIbS-Formulated Diet with Powdered Ipomoea batatas Shoots, FD+HWEIbS- Formulated Diet with Hot-Water Extract of Ipomoea batatas Shoots, FD+CEEIbS- Formulated Diet with Crude Ethanolic Extract of Ipomoea batatas Shoots presence of vitamins A, B, C, and B12 (Ekenyem, & Madubuike, 2006) which are required for blood production (Lim *et al.*, 2000) that contribute to the activation of the non-specific immunity mechanism in the fish.

The fish that received FD+CEEIbS ranked the highest in terms of Platelet, Plateletcrit and Platelet large cell count (Table 4). Platelets are responsible for wound healing and have important roles in inflammatory and immune responses (Quinn *et al.*, 2005).

The results from the experiments showed an increased survival in the fish that received formulated diets with I. batatas powder or extract, compared to the fish that received the baseline formulated diet only (Figure 1).

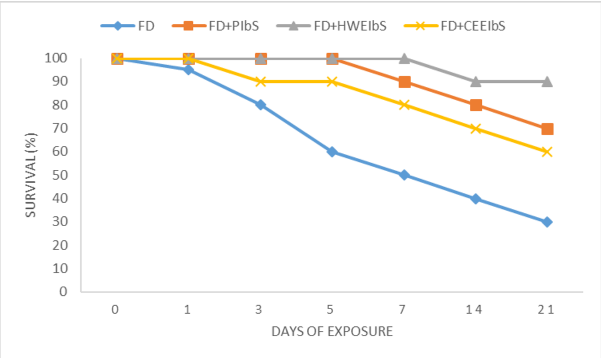

Figure 1. Survival of *O. niloticus* that received experimental feeds without or with *I. batatas* shoot powder or extract, and were then exposed to a sub-lethal dose of ammonium chloride for 21 d.

3.2 Behavioral and clinical signs and manifestations

Behavioral changes were observed during exposure of the Nile tilapia to ammonia. The swimming activity was reduced during the exposure, as a change in behavior. Within 24 hours of exposure, the control treatment shows mortality. Some fish that received FD+PIbS, FD+HWEIbS, and FD+CEEIbS displayed the same clinical signs throughout the study. However, the majority of the fish in these treatment groups exhibited normal behavior. The affected fish settled at the bottom of the aquarium and later moved upward to gasp for air. The mouth and gills of the deceased fish were open, and darkening of color was seen in the eyes and on the skin.

4. Conclusions

The present study aimed to assess the behavior, survival and haematological profile of tilapia fed with diets containing *I. batatas* powder or extracts, and then exposed to a sub-lethal dose of ammonia. The results show that the control treatment exhibited increased leukocyte indices during the exposure. Moreover, the erythrocyte indices of the fish treated with *I. batatas* were higher and significantly different from the control group. The fish that received FD+CEEIbS had considerably greater platelet (PLT) counts than in the other treatments. The fish that got FD+HWEIbS had the best survival rate among the dietary treatments. In conclusion, nutritional supplementation with *I. batatas*, particularly with its hot-water extract, enhanced tilapia's physiological resistance stress from a stressor such as ammonia, as evidenced by its haematological profile and high survival rate.

Table 3. Red blood cell and erythrocyte indices of tilapia after 21 d of exposure to ammonia

Treatment	Erythrocyte indices					
	Red blood cell (10^6/ml)	Hemoglobin (g/dL)	Hematocrit (%)	Mean corpuscular volume (fl)	Mean corpuscular haemoglobin (pg)	Mean corpuscular hemoglobin concentration (g/dL)
FD	1.55±0.04^a	64.33±1.91^a	21.25±0.73^a	137.55±4.33^b	42.20±0.63^a	299.00±4.92^a
FD+PIbS	1.75±0.08^a	81.33±4.86^b	28.32±1.17^b	162.58±6.00^a	46.27±1.35^a	286.50±10.36^a
FD+HWEIbS	1.59±0.18^a	71.67±9.66^a	26.17±3.71^a	162.40±7.06^a	44.65±1.46^a	276.50±6.97^b
FD+CEEIbS	1.48±0.08^a	65.33±4.80^a	23.07±1.51^a	155.75±3.03^a	43.93±1.07^a	282.33±5.52^a

Note: Means with different letters as superscripts are significantly different from each other *(*P< 0.05*)*. FD-Formulated Diet, FD+PIbS-Formulated Diet with Powdered Ipomoea batatas Shoots, FD+HWEIbS- Formulated Diet with Hot-Water Extract of Ipomoea batatas Shoots, FD+CEEIbS- Formulated Diet with Crude Ethanolic Extract of Ipomoea batatas Shoots

Table 4. Platelet and thrombocyte indices of tilapia after 21 d of exposure to ammonia

Treatment	Thrombocyte indices					
	Platelet (10^6/ml)	Mean platelet volume (fl)	Platelet distribution width (fl)	Plateletcrit (%)	Platelet large cell count (10^6/ml)	Platelet large cell ratio (%)
FD	21.33±1.05	16.07±0.30	7.83±0.46	0.03±0.00	9.33±0.42	46.67±0.48
FD+PIbS	13.83±5.95	20.18±2.74	8.68±1.11	0.02±0.01	6.50±2.70	47.67±12.40
FD+HWEIbS	23.00±6.85	14.72±0.92	7.50±0.37	0.03±0.01	10.67±3.12	47.05±13.08
FD+CEEIbS	31.83±4.71	14.45±1.12	8.75±0.94	0.04±0.00	12.50±1.06	44.37±5.65

Note: FD-Formulated Diet, FD+PIbS- Formulated Diet with Powdered Ipomoea batatas Shoots, FD+HWEIbS- Formulated Diet with Hot-Water Extract of Ipomoea batatas Shoots, FD+CEEIbS- Formulated Diet with Crude Ethanolic Extract of Ipomoea batatas Shoots

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