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Effect of Purple Sweet Potato Leaf Extract Supplementation on the Histopathology of Gills, Liver, and Kidneys in *Neolissochilus soroides* Exposed to Ammonia as a Stressor

Suciyono Suciyono[1, 2]*, Darmawan Setia Budi[1, 2], Hapsari Kenconojati[1, 2], Afri Arnita Sari[1], Shovia Finny Anggreani[1], Arafik Lamadi[3], Maya Nurwartanti Yunita[4], Ainulyakin H. Imlani[5]

[1]Aquaculture Study Program, Faculty of Health, Medicine and Life Science, Airlangga University, Banyuwangi Campus
[2]Sustainability Aquaculture and Environmental Research Group, Airlangga University, Indonesia
[3]Aquaculture Study Program, Faculty of Fisheries and Marine, Gorontalo University, 96128 Gorontalo, Indonesia
[4]Department of Veterinary Pathology, Faculty of Veterinary Medicine, Universitas Airlangga, Indonesia, Indonesia
[5]Tawi-Tawi College of Technology and Oceanography, Mindanao State University, Philippines
Corresponding author: suciyono@fpk.unair.ac.id

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ABSTRACT

This study investigated the potential effect of purple sweet potato (*Ipomoea batatas*) leaf extract as an antioxidant on the histopathological changes in the gills, liver, and kidneys of *Neolissochilus soroides* exposed to NH_4Cl. Over a 40-day supplementation period, four doses of the extract (0, 2.5, 5, and 7.5%) were administered with five replicates per dose. Following the supplementation, the fish were exposed to a 48-hour stressor of NH_4Cl at 10ppm. Histopathological observations were conducted at three time points: before rearing, after 40 days of supplementation, and after 48 hours of NH_4Cl exposure. The results revealed that purple sweet potato leaf extract supplementation significantly ($P< 0.05$) increased the number of normal cells in the liver and kidneys compared to the control group. The 5 and 7.5% doses showed no significant difference from each other but were significantly more effective than the 2.5% dose and the control. Furthermore, both the 5 and 7.5% doses significantly reduced cellular damage, such as necrosis, in the target organs induced by NH_4Cl exposure compared to the control. These findings suggest that higher doses of *Ipomoea batatas* leaf extract increase normal cell in the liver and kidneys while mitigating cellular damage caused by NH_4Cl stress. In conclusion, supplementation with purple sweet potato leaf extract provides a protective effect against organ damage in *N. soroides* under NH_4Cl-induced stress, with higher doses showing the most significant beneficial outcomes in terms of cell preservation and damage reduction.

INTRODUCTION

The ammonia is a major toxic stressor in aquaculture, affecting both aquatic organisms and their environments **(Parvathy *et al.*, 2023)**. This is due to the fish protein breakdown,

uneaten feed, algal blooms, and organic matter decomposition **(Nagaraju *et al.*, 2023; Edwards *et al.*, 2024)**. When ammonia levels rise, particularly in their unionized form (NH_3), they can quickly accumulate to lethal levels, interrupting nitrogen excretion, raising ammonia levels in the blood and tissues, and interfering with normal metabolic processes in fish **(Xu *et al.*, 2021)**. Furthermore, ammonia exposure can cause an excess of reactive oxygen species (ROS), resulting in cellular damage, including the breakdown of mitochondrial membranes **(Zhang *et al.* 2020)**. Ammonia toxicity, can seriously affect essential organs such as the gills, kidneys, and liver. Gills may degenerate and hemorrhage may occur, while the liver may exhibit pyknosis, nuclear enlargement, and vacuolization **(Esam *et al.*, 2022; Liang *et al.*, 2022)**. Ammonia exposure in the kidneys can cause structural damage, including abnormal tubules and cellular degeneration **(Mangang *et al.*, 2021)**. *N. soroides*, popularly known as the Mahseer or "God fish" in Indonesia, is one species that is particularly susceptible to ammonia poisoning **(Khaironizam *et al.*, 2015)**. This species is native to South and Southeast Asia and is highly valued in Indonesian local markets, with values ranging from IDR 350,000 to 1,500,000 per kilogram **(Putri *et al.*, 2020)**. Despite its economic value, the cultivation of *N. soroides* is limited due to its sensitivity to water quality fluctuations and habitat disturbances **(Khudamrongsawat *et al.*, 2021)**. One significant challenge in intensifying aquaculture for this species is the increased use of commercial feed, which contributes to ammonia pollution in aquaculture environments **(Wahyuningsih & Gitarama, 2020)**. To mitigate these risks, water quality management practices, including the use of probiotics, have been implemented **(Wahyuningsih & Gitarama, 2020)**.

In addition to these approaches, phytobiotic supplementation has emerged as a promising solution to improve fish resilience to stress, especially oxidative stress. Phytobiotics, rich in antioxidants, can help neutralize free radicals and reduce cellular damage **(Yilmaz, 2019; Forman & Zhang, 2021)**. One such promising phytobiotic is purple sweet potato leaf (*Ipomoea batatas*), which is abundant in phenolic compounds such as flavonoids and anthocyanins **(Alam, 2021)**. These compounds have demonstrated antioxidant and immune-boosting effects in fish, offering protection against oxidative stress and improving survival rates, particularly under ammonia exposure **(Yilmaz, 2019; Elaziz *et al.*, 2023)**. Previous studies have shown the beneficial effects of *I. batatas* leaf extract on the hematological profile of the Nile tilapia **(Baldo *et al.*, 2022)** and its ability to mitigate oxidative stress in catfish **(Tomori *et al.*, 2022)**.

However, to date, no research has evaluated the impact of *I. batatas* leaf extract supplementation on the histopathological changes in *N. soroides* exposed to ammonia (NH_4Cl). The aim of this study was to investigate whether supplementation with purple sweet potato leaf extract can influence the histopathological profiles of the gills, kidneys, and liver in *N. soroides* exposed to ammonia as a stressor. This research could provide valuable insights into the potential therapeutic effects of phytobiotics in mitigating the adverse impacts of ammonia toxicity in aquaculture systems.

MATERIALS AND METHODS

Preparation

Origin of I. batatas leaves

I. batatas leaves used were obtained from local farmers (Banyuwangi, Indonesia), and were cleaned of dirt. Next, the leaves were air-dried at about 25°C (five days). After drying, the leaves were ground using a Getra Spice Herb Grinder IC-04A (Indonesia) in batches and were sieved with a filter (mesh size 100) to obtain a finer and more uniform powder, then stored in an airtight container and in a dry and cool environment.

I. batatas extract preparation

The extraction stage of sweet potato leaf extraction was carried out by soaking 500 grams of purple sweet potato leaf powder in 5 liters of 70% ethanol (ROFA laboratory Center, Indonesia) for 72 hours **(Baldo *et al.*, 2022)**. 70% ethanol solvent was used since it obtains the highest anthocyanin and flavonoid content, as outlined by **Nguyen *et al.* (2021)**. Then, the sample was filtered using Whatman paper no.42. The filtrate was then separated from the solvent, ethanol, using a rotary evaporator (IKA RV 10 digital V, Germany) at 45ºC with a speed of 100rpm. The liquid extract that has been separated from ethanol was then stored in a refrigerator at 4ºC. The extract was then sprayed evenly on the feed and air-dried to dry before storage **(Ariyanti *et al.*, 2022)**.

Fish rearing

A total of 200 *N. soroides* with an average length and weight of 13.30 ± 1.34cm and 28.66 ± 7.85g, respectively, were used in this study. A completely randomized design with 4 treatments and 5 replicates was employed, where the control treatment (T1) was reared without sweet potato leaf extract supplementation; Treatment 2 was supplemented with 2.5% sweet potato leaf extract; Treatment 3 was supplemented with 5% sweet potato leaf extract; and Treatment 4 was supplemented with 7.5% sweet potato leaf extract. Each treatment was maintained for 40 days at a density of 10 individuals/aquarium (54 L) with an *ad libitum* feeding method (Hi-Pro-Vite, 33% protein, Indonesia), twice a day. Furthermore, each treatment was exposed to NH_4Cl (Pro analysis, Merck, Germany) following the procedure of **Li *et al.* (2019)** at a dose of 10ppm, which refers to the sublethal concentration (LC_{50}) in *N. soroides* in the previous study.

Tissue sampling

Organ samples observed in this study were gills, kidneys, and liver. The three organ samples were observed before rearing, after 40 days of treatment with *I. Batatas* extract supplementation, and 48 hours after exposure to ammonia stressor. All organs were preserved using 10% Buffered Neutral Formalin (BNF) solution to maintain tissue integrity. Subsequently, histology preparations were made with hematoxilin eosin (HE) staining at the Animal Health Clinic Laboratory in Malang.

Observed parameters

Calculations were carried out using a semi-quantitative method, and this method was determined using morphological criteria such as cells that experience inflammation, necrosis, or other damage **(Meyerholz & Beck, 2018)**. Furthermore, the calculation formula for each cell morphology criterion (normal cells, degenerated cells, hemorrhage, and necrosis) was applied to five fields of view for each sample in every treatment **(Indriana *et al.*, 2020)**, following the specified formula:

$$P\% = \frac{\sum KS}{\sum TS} \times 100$$

Where:

P (%) = Percentage of normal cells/degenerated cells/haemorrhaged cells/necrosis cells

$\sum$ KS = Total number of normal cells/degenerated cells/haemorrhaged cells/necrosis cells

$\sum$ TS = Total number of cells

Data analysis

The effect of supplementation of *I. batata* extract on the number of normal cells, necrosis, and hemorrhage in the three organs was analyzed using ANOVA (Analysis of Variance) with a confidence level of 95% ($\alpha = 0.05$). Furthermore, to determine the significant effect of each dose of supplementation of *I. batata* extract on the parameters observed, further tests were implemented with the Duncan Multiple Range Test (DMRT).

RESULTS

Histopathology analysis revealed degeneration, hemorrhage, and necrosis in the gills, kidneys, and liver of *N. soroides* (Fig. 1). Degeneration of the gill organs was marked by edema or inflammation. Hemorrhage was distinguished by the dark red coloring of muscle fibers caused by the creation of blood cells. The blood vessels contained empty spaces. Necrosis was distinguished by swelling or inflammation. Degeneration in the kidney and liver included structural alterations such as edema or inflammation. A hemorrhage was defined as bleeding within or around an organ, as well as coloring to red or blackish. Necrosis in fish kidneys and liver was distinguished by yellowish or white coloration, edema, or inflammation.

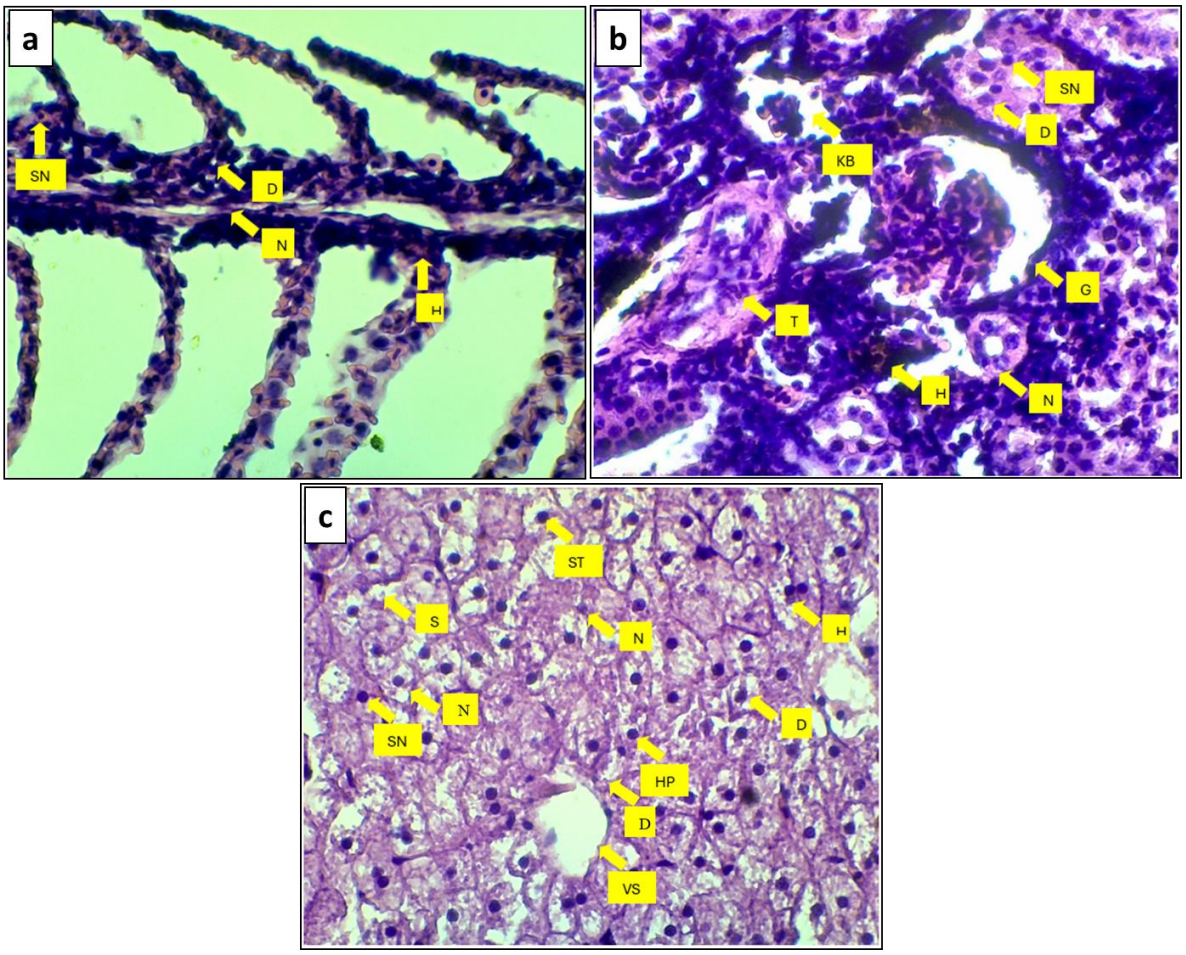

Fig. 1. Histopathology of Mahseer (*N. soroides*) organ with HE staining. Magnification: 400x. Scale bar: 20µm. (**a: Gill, b: kidney, c: liver**). Description: SN = Normal Cells, D = Degeneration, H = Hemorrhage, N = Necrosis. G = Glomerulus, T = Tubules, KB = Bowman's Capsule, HP = Hepatocytes, ST = Cytoplasm, VS = Central Vein, S = Sinusoids

- **Normal cells**

Supplementation of *I. batata* leaf extract in the feed for 40 days had a significant effect ($P< 0.05$) on the increase of normal cells in three organs (gills, kidneys, and liver) of *N. soroides* (Fig. 2). The highest increase in normal cell count was observed in the treatment with a 7.5% dose (T4), followed by a 5% dose (T3). However, no significant difference ($P> 0.05$) was found between these two doses. Both treatments showed significant differences compared to the 2.5% sweet potato leaf extract dose (T2) and the control group. After exposure to NH_4Cl for 48 hours, no significant differences were found across all treatments. The normal cell values for each treatment are shown in Fig. (1).

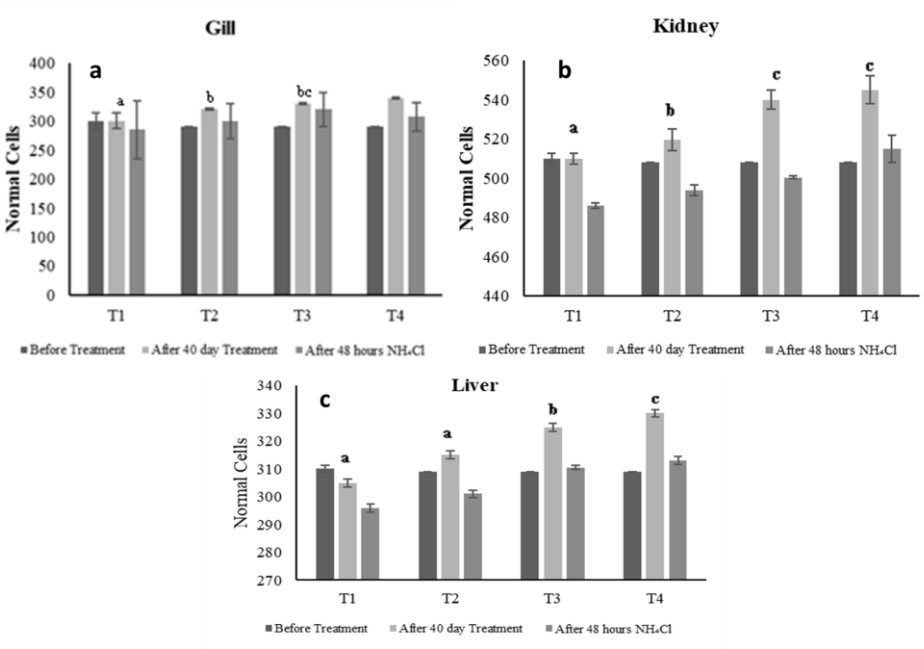

Fig. 2. The normal cell values in the histology preparations of *N. soroides* (cells taken from five fields of view) were observed before maintenance, after 40 days of maintenance, and 48 hours after exposure to 10 ppm NH_4Cl

- **Tissue damage to the *N. soroides* organ**

Supplementing *I. batata* extract with feed significantly reduced cell degeneration (Fig. 3), bleeding (Fig. 4), and necrosis (Fig. 5) in the gill, kidney, and liver organs after 40 days of maintenance ($P< 0.05$).

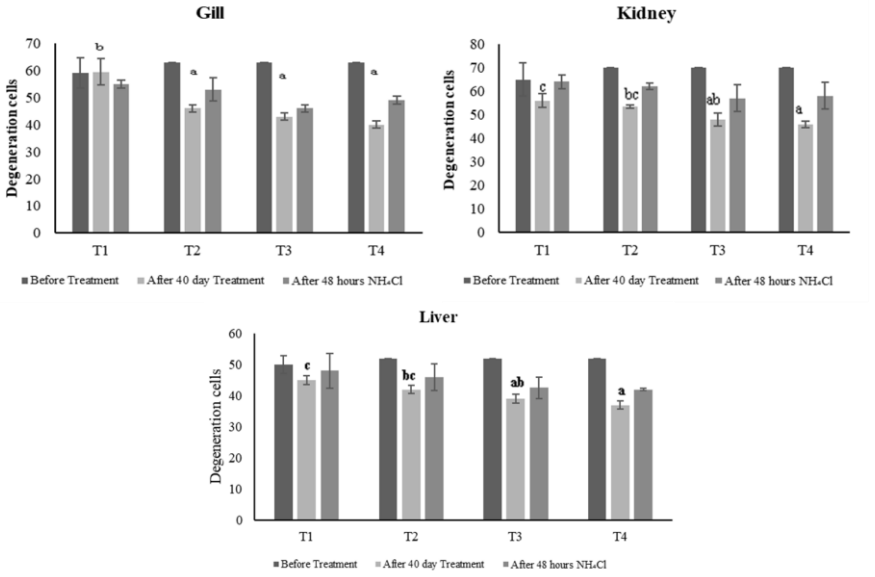

Fig. 3. The degenerative cell values in the histology preparations of *N. soroides* (cells taken from five fields of view) were observed before maintenance, after 40 days of maintenance, and 48 hours after exposure to 10ppm NH_4Cl

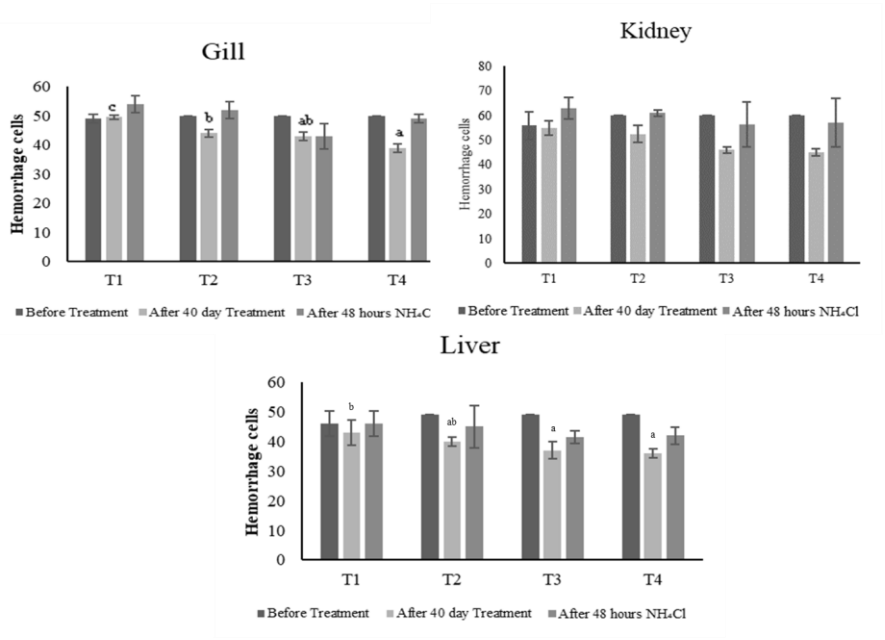

Fig. 4. The hemorrhage cell values in the histology preparations of *N. soroides* (cells taken from five fields of view) were observed before maintenance, after 40 days of maintenance, and 48 hours after exposure to 10ppm NH_4Cl

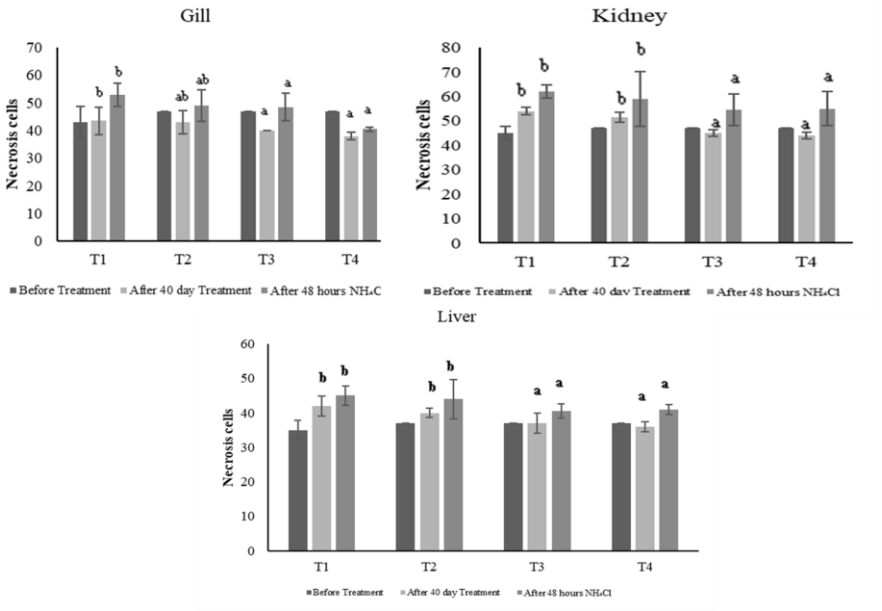

Fig. 5. The necrosis cell values in the histology preparations of *N. soroides* (cells taken from five fields of view) were observed before maintenance, after 40 days of maintenance, and 48 hours after exposure to 10ppm NH_4Cl

The 7.5% extract of *I. batata* was the most effective at reducing cell damage, hemorrhage, and degeneration. Furthermore, 7.5 and 5% extracts of *I. batata* were more

effective in suppressing cell necrosis than the 2.5% dosage treatment and control group. However, following 48 hours of exposure to NH_4Cl, there was no significant difference ($P > 0.05$) in the number of deteriorated and hemorrhaged cells across all treatments. However, the lowest necrosis was successively reported at doses of 7.5, 5, and 2.5%, respectively.

DISCUSSION

Supplementation of *I. batatas* leaf extract was able to increase the number of normal cells in gill, kidney, and liver organs in *N. soroides*. The increase in the number of normal cells increased as the dose of *I. batatas* applied during maintenance (40 days) increased. Furthermore, this extract demonstrated the ability to suppress cell damage in organs following NH4Cl exposure. As the dose increased, the extent of cell damage decreased. This indicates that *I. batata* leaf extract is able to improve the health of *N. soroides* fish. *I. batata* contains phytochemical compounds (flavonoids and anthocyanins), which are antioxidants that can reduce oxidative stress, eliminate free radicals, and be anti-stress **(de Rezende *et al.*, 2021)**. Oxidative stress triggers an increase in reactive oxygen species (ROS) which in excessive amounts will disrupt cell homeostasis and will increase macromolecular damage **(Paredes *et al.*, 2019)**. The fish defense system consists of two ways, namely enzymatic antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione stransferase (GST), glutathione reductase (GRd), and non-enzyme antioxidants, in the form of organic molecules derived from phytochemicals and vitamins **(Moussa *et al.*, 2019; Hoseinifar *et al.*, 2020)**. Flavonoids as non-enzymatic antioxidants can prevent cell damage caused by free radicals through ROS uptake, antioxidant enzyme activation, and oxidase inhibition **(Shen *et al.*, 2022)**.

Furthermore, anthocyanin compounds in *I. batata* function to increase the number of monocyte cells **(Chen *et al.*, 2023)**. Monocytes function in removing cellular debris from tissue necrosis due to inflammation **(da Silva *et al.*, 2018)**. That way, normal cells can quickly form to replace damaged cells. This finding is in line with the research of **Baldo *et al.* (2022)**, which shows that sweet potato leaf extract added to feed can increase normal liver cells exposed to ammonia. Exposure to NH_4Cl for 48 hours in this study affects degeneration, haemorrhage, and necrosis in the gills, kidneys, and liver of *N. soroides* fish, where the most damage occurs in the gills. These three organs play a role in defence against environmental toxicity, against water pollutants such as ammonia **(Xu *et al.*, 2021)**.

When fish are exposed to non-lethal or lethal ammonia, high ammonia stress leads to increased blood cortisol levels and oxidative stress in the gills **(Jahanbani *et al.*, 2023)**. The gills as the first organ exposed to stressors in the environment will experience primary and secondary lamellar degeneration and vascular congestion **(Esam *et al.*, 2022)**. On the other hand, ammonia nitrogen exposure to the liver leads to a decrease in cytoplasmic glycogen or even vacuole formation, usually with changes including

oedematous degeneration, vacuolisation, and local necrosis **(Xu *et al.*, 2021)**. Whereas, ammonia exposure to the kidney will induce ROS formation, causing an inflammatory response and oxidative stress response leading to apoptosis in renal head macrophages **(He *et al.*, 2021)**.

Supplementation of 5 and 7.5% doses of *I. batata* extract was shown to reduce the number of necrosis of *N. soroides* exposed to NH_4CL. *I. batata* acts as an anti-inflammatory due to the content of plofenols (phenolic acids and flavonoids) which can inhibit pro-inflammatory enzymes reducing the production of inflammatory cytokines and inhibit protein denaturation **(Bashir *et al.*, 2023; Islam, 2024)**. In addition, supplementation of *I. batata* leaf extract can improve the histopathological structure of the gills, increase the number of epithelial cells, and reduce the number of inflammatory cells **(Mahfudh *et al.*, 2022; Artini *et al.*, 2022)**.

CONCLUSION

Supplementation of *I. batata* leaf extract had a positive effect on increasing immunity and was able to reduce the level of organ damage to *N. soroides* fish from NH_4Cl exposure. The higher the dose application of *I. batata* extract, the lower the level of organ damage. The best dose in this study was 7.5%, followed by 5% *I. patata* extract. Phenolic compounds, flavonoids, and anthocyanins contained in the extract play an important role in cell maintenance and protect body cells from oxidative stress damage. The decrease in cell damage, including necrosis, after NH_4Cl exposure indicated that the purple sweet potato leaf extract was not only effective in reducing the harmful effects of reactive oxygen species (ROS) but also had significant anti-inflammatory properties, which helped reduce further tissue damage.

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Conflict of interest

All authors declare that there is no conflict of interest in this research

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