



Ameliorating deleterious effects of ammonia toxicity on *Oreochromis niloticus* using *Yucca schidigera* extract as a water supplement

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ARTICLE INFO

Keywords:

Yucca schidigera extract
Ammonia
Growth
Immunity
Fish

ABSTRACT

The current study attempted to establish *Yucca schidigera* extract (YSE) modifying effects on long-term ammonia (NH₃) poisoning in Nile Tilapia. Randomly, 300 Nile tilapia fish were divided into 5 equal groups, each including four replications. One group was retained as the standard control (1st) group, and the 2nd group was subjected to NH₃ for three weeks (during the last week of the 1st, 2nd and 3rd month of the experiment), and the 3rd, 4th and 5th groups were supplemented with YSE (6, 8 and 10 mg/L water every 2 days) and subjected to NH₃ for three weeks (during the last week of the 1st, 2nd and the 3rd month of the experiment), respectively. A significant improvement in water quality was recorded with YSE by increasing dissolved oxygen in the water and decreasing water pH, total ammonia nitrogen concentration, and un-ionized ammonia. Feed intake and feed conversion were improved with all groups supplemented with YSE. YSE supplementation to ammonia-polluted water of Nile tilapia significantly improved liver and kidney functions such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), creatinine, urea, and uric acid. YSE improved immunity and antioxidant parameters when compared to the ammonia group. Digestive enzymes were enhanced by the addition of YSE to the ammonia-polluted water of Nile tilapia. In conclusion, our investigation aimed to declare YSE modulatory effects on the quality of water, growth performance, immunological status, and antioxidant capacity in fish.

1. Introduction

The aquaculture industry's most effective strategy for meeting the human need for protein and reducing strain on wild fisheries resources is to intensify fish farming (Aubin et al., 2019). As fish numbers increase, more feed is consumed, and thus more nitrogen is released, which can affect the surrounding water bodies and fish metabolism (Besson et al., 2016; Rizwanuddin et al., 2023). Thus, the enhancement decreases nitrogen excretion and protein metabolism, which have unavoidable implications for developing the sustainable aquaculture industry (Kelly and Kohler, 2003). The feed additives from nature, such as prebiotics

and beneficial microorganisms and phytobiotics) as well as artificial materials (growth-promoting agents, and antibiotics), were incorporated into fish food to enhance fish development, feed utilization, immune system and physiology, and disease resistance (Ali et al., 2016; Reda et al., 2020; El Asely et al., 2020; Elbially et al., 2021; Ullah et al., 2023). Furthermore, functional herbal substances, such as water or feed additives, are strongly recommended as eco-friendly additives (Alagawany et al., 2020; Abdel-Tawwab et al., 2021; Saleem et al., 2024a,b). YSE is a rich supply of chemicals known as saponins, such as glycoside and steroidal saponin fractions (Ayasan et al., 2005; Adnan et al., 2022). The components of YSE active have been utilized primarily

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<https://doi.org/10.1016/j.aqrep.2025.102673>

Received 12 October 2024; Received in revised form 3 January 2025; Accepted 6 February 2025

Available online 11 February 2025

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to reduce ammonia in cattle, poultry, and fish excretion as a byproduct of the metabolism of proteins and decrease animal contamination of the ambient environment (El-Saidy and Gaber, 2004). YSE comprises two active substances: a glycocomponent part that binds to ammonia and a steroidal saponin part with surface active characteristics. Ayasan et al. (2005) mentioned how saponins enhance an animal's immunity and performance. Several studies found that the performances of aquatic animals showed improvements as a direct result of using YSE as water or feed additives (Abdel-Tawwab et al., 2021; Paray et al., 2021). In both fresh and salt water, the direct integration of YSE into aquatic systems for animal rearing has shown a favorable impact on lowering ammonia concentration in a dose-reliant way.

On the other hand, according to Khalil et al. (2015) and Fayed et al. (2019), YSE is a possibly safe material appropriate for controlling water quality and lowering ammonia levels. Additionally, at a dose of 0.75 mg kg⁻¹ diet, dietary supplementation of YSE dramatically enhanced Nile tilapia growth performance and enhanced its consumption of plant-derived protein to fulfill the nutritional requirements (Gaber, 2006). On the other hand, using 1.0 g/L of both YE + yeast as water additives improved water quality by reducing levels of ammonia and pH, which appears to enhance the growth rate and reduce the oxidative stress in Nile tilapia (Abdel-Tawwab et al., 2021). YSE was added to the fish feed to boost protein digestion and metabolism and decrease ammonia elimination (El-Saidy et al., 2004; Gaber, 2006). YSE may be used in aquaculture, similar to its use in terrestrial animals. Much research on using *Y. schidigera* in fish feeds has been conducted. These include studies on the Nile tilapia (Gaber, 2006), channel catfish (Kelly and Kohler, 2003), and hybrid tilapia (Kelly and Kohler, 2003). According to studies conducted by Cheeke (2000), YSE has been effectively utilized to suppress the buildup of ammonium in animal housing and lower the amount of ammonium in water and the smell of feces in animal waste. In fresh and saltwater fish waste, *Y. schidigera* can reduce the accumulation of ammonia from biogenic sources, hydrogen sulfide, and other chemicals that are detrimental to fish health (Santacruz-Reyes and Chien, 2010). The literature about applying YSE as a water treatment in aquaculture to mitigate the stressors induced by ecological pollutants is limited. This investigation seeks to evaluate the impact of YSE on mitigating the detrimental effects of ammonia on the performance, physiological status, immunological characteristics, and antioxidant activity of Nile tilapia.

2. Material and methods

2.1. Preparation of ammonium solution and *Yucca schidigera* extract

An ammonium solution was prepared and kept to 5 mg/L. A stock solution (5 mg/L) was prepared daily by mixing NH₄Cl with dechlorinated tap water. YSE (3 % saponins; ANOVA Pharm Company, Tanta, Egypt) was supplied every two days at a rate of 8 mg/L in water.

2.2. Fish management and diet formulation

Healthy Nile tilapia specimens with an average body weight of 4.00 ± 0.05 g were acquired from the Fish Hatchery of the Central Laboratory for Aquaculture Research (CLAR) in Abbassa, Egypt. Following a 14-day acclimatization phase, fish were randomly distributed into glass aquaria with 70 L of chloride-free tap water. Each container was equipped with an air pump linked to a central air compressor to ensure continual aeration. Fish excrement was extracted from each container, and the water was partially replaced daily (about 30 %). The water quality parameters were maintained at 27.6–29.6 °C for temperature, 7.50–8.0 mg/L for dissolved oxygen (DO), 7.40–8.1 for pH values, 0.01–0.03 mg/L for nitrite levels, 0.01–0.04 mg/L for nitrate levels, and 0.1–0.3 mg/L for the un-ionized ammonia. The fish were maintained under optimal conditions with a 12-h light and 12-h dark cycle. The basal diet was designed to satisfy the nutritional needs of the

fish (NRC, 2011). The mathematical and chemical analysis of the basal ration is presented in Table 1. Fish were nourished thrice daily (9:00 am, 12:00 pm, and 4:00 pm) at a rate of 5 % of their live body weight. Throughout the trial duration, the feed intake for each replicate was recalibrated biweekly based on biomass measurements.

2.3. Experimental design

Three hundred Nile tilapia fish were randomly divided into five equal groups (4 replicates/group) and treated for 3 months. Group 1 was the control; group 2 received ammonia (NH₃) the fourth week of each month; groups 3, 4, and 5 were supplemented with YSE (6, 8, and 10 mg/L water every 2 days, respectively) and received NH₃ the fourth week of each month. The research was done in the Faculty of Agriculture at Zagazig University, Egypt. The Ethics of Animal Use in Research Committee (EAURC) at Zagazig University, Egypt, approved the animal care and experimental procedures (ZU-IACUC/2/F/110/2022).

2.4. Water quality measurement

Water quality metrics in each aquarium were documented weekly throughout the trial. Total ammonia and pH concentration were measured using the Hach Kit model HI 83,205 (Multiparameter Bench Photometer, Hanna Instruments, Romania). The HI 9146 Oxygen and Temperature Meter (Hanna Instruments, Romania) quantified dissolved oxygen and temperature.

2.5. Growth performance parameters

Every two weeks, fish weights and counts were documented to evaluate growth performance. The following formulas were used to determine body weight gain, feed conversion ratio (FCR), specific

Table 1

Formulation of the basal diet fed to experimental *Oreochromis niloticus* fish.

Ingredient	Control diet
Ingredients %	
Fish meal (72 % CP)	12
Soybean meal (44 % CP)	37
Yellow corn (8.5 % CP)	28
Rice bran (12.9 % CP)	10
Wheat bran (15.7 % CP)	10
Soybean oil	2
Vitamins	0.5
Minerals	0.5
Total	100
Chemical composition (% DM)	
Dry matter	89.87
Organic matter	83.91
Crude protein	30.29
Ether extract	5.03
Crude fiber	6.70
Crude ash	5.96
Nitrogen Free Extract ^a	43.13
Calculated energy value	
GE (Kcal/kg) ^b	429
DE (Kcal/Kg) ^c	321
CP/GE (mg/Kcal) ^d	706

^a Nitrogen Free Extract was calculated by the difference: 100 - (moisture + protein + lipid + ash + Crude fiber).

^b GE (Gross energy) was calculated according to NRC (2011) by using factors of 5.65, 9.45 and 4.22 K cal per gram of protein, lipid and carbohydrate, respectively.

^c DE (Digestible energy) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy according to Hepher et al. (1983).

^d P/E (protein energy ratio) = crude protein x 10,000 / digestible energy, according to Hepher et al. (1983).

growth rate (SGR), and survival rates (SR):

$$\text{FCR} = (\text{Feed intake, g}) / (\text{Weight gain, g}).$$

$$\text{SGR} = [\text{final mean body weight} - \text{initial mean body weight}] \times 100.$$

$$\text{SR} = (\text{no. survived/no. stocked}) \times 100.$$

2.6. Sample collection

Blood samples were obtained from the caudal veins of fish (5 fish per replicate) using heparinized syringes and gathered in plastic tubes. Each sample was separated into two portions; one was utilized for rapid hematological studies, while the other was centrifuged for 20 min at 3000 rpm for plasma extraction. The plasma samples were preserved at -20°C until biochemical analysis was conducted.

2.7. Hematology and immunity biomarkers

The total red blood cell count (RBCs), leukocyte count (WBCs), hemoglobin (Hb) concentration, and hematocrit value (PCV) were assessed with a Sysmex XN-2000 automated hematology analyzer (Sysmex America Inc., USA). Plasma levels of immunoglobulin G (IgG-Cataltlog no. MBS2800419), A (IgA-Cataltlog no. MBS034250), and M (IgM-Cataltlog no. MBS700823) were measured using commercial ELISA kits from Aquatic Diagnostics Ltd, Scotland, and the UK. Plasma lysozyme (Cataltlog no. MBS099538) activity was assessed by spectrophotometry, according to Ellis (1990). Complement 3 (C3) was quantified using commercial fish ELISA kits from My BioSource, San Diego, USA (Catalogue No. MBS042385). Nitric oxide (NO) was quantified colorimetrically using the kit from Bio Diagnostics Co., Egypt.

2.8. Liver and kidney functions

Using commercial kits produced by Diamond Diagnostics Company, Egypt, the following parameters were measured: total protein (TP), globulin, albumin, urea, creatinine, uric acid, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and plasma levels of transaminase enzymes (AST; aspartate aminotransferase and ALT; alanine aminotransferase).

2.9. Antioxidants and lipid peroxidation markers

The activities of superoxide dismutase (SOD) and catalase (CAT), along with the amount of reduced glutathione, were assessed using commercial kits according to the manufacturer's guidelines (Catalog no. MBS705758, MBS705697, and GSH-MBS8807785, respectively). TAC was evaluated following the manufacturer's instructions utilizing the kit from Biodiagnostic Company, Egypt Catalogue Number TA 25 13. Ohkawa et al. (1979) colorimetric approach was used to test malondialdehyde (MDA).

2.10. Amylase and lipase activities

According to Bernfeld (1955), the evaluation of amylase activity was carried out by incubating a crude extract of the intestine (60 μL) with a 2% starch solution (375 μL) and a 10 mM phosphate buffer with a pH of 8.0 (375 μL) that included 0.9% sodium chloride (w/v) at a temperature of 25°C . Twenty minutes later, 3, 5-dinitro salicylic acid (DNSA) was added to the solution, and then it was heated at 100 degrees Celsius for ten minutes. Following the equilibration of the temperature, the absorbance was measured at 570 nm against a blank that was generated similarly, except that the crude extract sample was substituted with 10 mM phosphate buffer. According to the definition, one unit of enzymatic activity is the quantity of enzyme necessary to hydrolyze 1 μg of maltose per mg of protein per minute, referred to as specific activity. A modified version of the approach developed by Aryee et al. (2007) was utilized to determine lipase activity. The substrate stock solution

consisted of 8 mM p-nitrophenyl palmitate (p-NPP) dissolved in 2-propanol. To prepare the substrate solution, one milliliter of the stock solution was dissolved in nine milliliters of fifty millimolar Tris-HCl containing 0.4% (w/v) Tween-80 and 0.1% (w/v) Arabic gum at a pH of eight, which is referred to as TTA buffer, pH eight. After 20 min, a microplate reader was utilized to determine the rate of p-NPP hydrolysis at 410 nm wavelength. To perform the samples, 100 μL of intestinal crude extract was added to 900 μL of substrate. The blanks were also prepared using 0.15 M NaCl solution instead of crude extract. Under the circumstances of the experiment, the quantity of enzyme that catalyzed the release of one micromole of p-nitrophenol (p-NP) per minute was considered one unit of activity (U). It was represented as U mg^{-1} of protein, the specific activity.

2.11. Statistical analysis

The SAS (2002) was used for statistical analysis. Immunity, lipid profile, antioxidant indices, liver and kidney functions, performance, and water quality were evaluated using a one-way ANOVA followed by the Tukey's test. A $P < 0.05$ was considered significant.

3. Results

3.1. Water quality

The effect of YSE supplementation (6, 8, and 10 mg/L water every two days) against NH_3 pollution (5 mg/L) on the water quality of *O. niloticus* is illustrated in Table 2. The results illustrate a significant increase in groups 2 ($P < 0.001$) and 3 ($P < 0.01$) if compared to groups 1, 4 and 5 and a significant decrease in pH in groups 4 and 5 ($P < 0.001$) compared to group 1, 2 and 3. Additionally, total ammonia nitrogen concentration (TAN) and un-ionized ammonia (UIA) were significantly increased in all groups ($P < 0.001$) if compared to the group 1. Nevertheless, a significant mitigation was observed in groups 4 and 5 ($P < 0.001$) if compared to groups 2 and 3. It worth noting that, group 5 reached a UIA concentration even lower than group 1, although not significant.

3.2. Growth performance

Table 3 displays Nile tilapia's growth performance at different YSE dosages. Compared to the other groups, the FW, DWG, SGR of Nile tilapia was significantly reduced in group 2 ($P < 0.001$), while significantly increased in a dose-dependent manner in groups 3, 4 and 5 with respect to group 2 ($P < 0.001$). Nevertheless, only group 5 reached a value significantly higher than group 1 ($P < 0.001$). As for DFI and SR, groups 2, 3 and 4 showed a significant reduction if compared to groups 1 and 5 ($P < 0.001$), while group 5 showed a significant increase with respect to the other groups ($P < 0.001$ and $P < 0.05$, respectively). Conversely, FCR showed a significant increase in group 2 with respect to the other groups ($P < 0.001$), and a significant reduction in group 5 compared to the other groups ($P < 0.001$).

3.3. Hematology and oxidative stress

The impact of supplementing YSE on hematological and oxidative stress parameters of *O. niloticus* in the presence of NH_3 (5 mg/L) pollution is illustrated in Table 4. The finding demonstrated a significant increase in RBC, WBC and glucose in groups 3, 4 and 5 with respect to group 1 and 2 ($P < 0.001$), with the only exception of group 3 with respect to group 2 ($P < 0.01$) for RBC. A similar trend was observed with cortisol and NO. YSE supplementation significantly decreased both parameters in groups 3, 4, and 5 with respect to group 1 and 2 ($P < 0.001$), with the only exception of group 5 with respect to group 1 ($P < 0.05$) for NO.

Table 2

Effect of *Yucca schidigera extract* (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on water quality ($\bar{X} \pm SE^1$) of *O. niloticus*.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
Temperature (°C)	29.65 ± 0.07	29.93 ± 0.14	29.77 ± 0.22	29.85 ± 0.18	29.87 ± 0.22	0.8216
Dissolved oxygen (mg L ⁻¹)	5.88 ± 0.18	5.41 ± 0.14	5.34 ± 0.16	5.54 ± 0.13	5.93 ± 0.13	0.0590
pH	7.85 ± 0.09 ^{abc}	8.09 ± 0.06 ^a	7.92 ± 0.07 ^{ab}	7.66 ± 0.08 ^{bc}	7.62 ± 0.09 ^c	< 0.001
TAN (mg/L)	0.30 ± 0.05 ^c	5.86 ± 0.10 ^a	2.34 ± 0.09 ^b	1.81 ± 0.06 ^b	0.62 ± 0.07 ^b	< 0.001
UAI (mg/L)	0.018 ± 0.001 ^d	0.36 ± 0.04 ^a	0.24 ± 0.016 ^b	0.15 ± 0.008 ^c	0.03 ± 0.004 ^d	< 0.001

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

TAN, Total ammonia nitrogen concentration; UAI, un-ionized ammonia.

¹SE: standard error.

Table 3

Effect of *Yucca schidigera extract* (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on performance ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters ^b	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
IW (g fish ⁻¹)	16.52 ± 0.13	16.54 ± 0.12	16.55 ± 0.17	16.51 ± 0.17	16.52 ± 0.18	0.9996
FW (g fish ⁻¹)	58.84 ± 1.88 ^{ab}	47.98 ± 1.46 ^c	54.95 ± 1.82 ^b	58.22 ± 2.09 ^{ab}	63.63 ± 1.70 ^a	< 0.001
DWG (g fish ⁻¹)	0.50 ± 0.02 ^{ab}	0.37 ± 0.02 ^c	0.46 ± 0.02 ^b	0.50 ± 0.03 ^{ab}	0.56 ± 0.02 ^a	< 0.01
SGR (%)	1.51 ± 0.03 ^{ab}	1.27 ± 0.04 ^c	1.43 ± 0.03 ^b	1.50 ± 0.06 ^{ab}	1.60 ± 0.03 ^a	< 0.01
DFI (g fish ⁻¹)	1.05 ± 0.01 ^{ab}	0.90 ± 0.01 ^d	0.96 ± 0.01 ^c	1.02 ± 0.01 ^b	1.07 ± 0.01 ^a	< 0.001
FCR (g/g)	2.08 ± 0.06 ^b	2.43 ± 0.11 ^a	2.11 ± 0.08 ^b	2.07 ± 0.10 ^b	1.92 ± 0.05 ^b	< 0.05
Survival rate (%)	98.89 ± 1.13 ^a	85.56 ± 2.22 ^c	92.22 ± 1.11 ^b	95.56 ± 1.15 ^{ab}	97.78 ± 1.13 ^a	< 0.001

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

^a SE: standard error.

^b IW, initial weight; FW, final weight; DWG, daily weight gain; SGR, specific growth rate; DFI, daily feed intake; FCR, feed conversion ratio; FCR: feed intake/weight gain.

Table 4

Effect of *Yucca schidigera extract* (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on hematology and oxidative stress ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters ^b	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
Hb (g/dl)	9.25 ± 0.26 ^a	7.36 ± 0.32 ^c	8.08 ± 0.36 ^{bc}	8.61 ± 0.19 ^{ab}	9.32 ± 0.19 ^a	< 0.01
RBCs (10 ⁶ /cmm)	3.57 ± 0.13 ^a	3.04 ± 0.10 ^c	3.14 ± 0.11 ^{bc}	3.19 ± 0.10 ^{bc}	3.43 ± 0.13 ^{ab}	< 0.05
WBCs (10 ³ /cmm)	17.45 ± 1.05 ^c	28.87 ± 1.52 ^a	24.52 ± 1.29 ^b	20.58 ± 1.55 ^{bc}	19.78 ± 1.36 ^c	< 0.01
Glucose (mg/dL)	80.75 ± 4.45 ^d	172.39 ± 11.41 ^a	131.12 ± 6.07 ^b	108.11 ± 7.92 ^{bc}	94.45 ± 5.30 ^{cd}	< 0.001
Cortisol (nmol/L)	34.11 ± 2.96 ^c	91.89 ± 5.10 ^a	69.25 ± 4.21 ^b	34.85 ± 2.30 ^c	40.08 ± 2.80 ^c	< 0.001
Nitric oxide (μmol/l)	0.31 ± 0.02 ^c	0.59 ± 0.04 ^a	0.43 ± 0.02 ^b	0.39 ± 0.03 ^{bc}	0.29 ± 0.02 ^c	< 0.001

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

^a SE: standard error.

^b Hb: hemoglobin, RBCs: red blood cell count, WBC: white blood cell count, PCV: content and hematocrit value.

3.4. Liver and kidney functions

The effect of YSE supplementation on the liver and kidney functions of *O. niloticus* in response to NH₃ contamination (5 mg/L) is illustrated in Table 5. The result demonstrated a significant increase in all parameters

of group 2 with respect to group 1 (P < 0.001) and a significant mitigating effect of the different YSE concentrations (P < 0.001), except for the A/G ratio in group 3, tending to reach the control values in a dose-dependent manner.

Table 5

Effect of *Yucca schidigera extract* (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on liver and kidney functions ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters ^b	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
Total protein (g/dL)	4.32 ± 0.11 ^{ab}	3.26 ± 0.22 ^d	3.55 ± 0.12 ^{cd}	3.90 ± 0.28 ^{bc}	4.57 ± 0.14 ^a	< 0.05
Albumin (g/dL)	2.57 ± 0.14	1.87 ± 0.13	2.21 ± 0.16	2.36 ± 0.27	2.53 ± 0.13	0.0940
Globulin (g/dL)	1.75 ± 0.11 ^{ab}	1.39 ± 0.12 ^c	1.34 ± 0.12 ^c	1.55 ± 0.05 ^{bc}	2.03 ± 0.11 ^a	< 0.01
A/G ratio ³	1.81 ± 0.13	2.05 ± 0.34	1.88 ± 0.21	1.77 ± 0.19	2.31 ± 0.27	0.5084
ALT (U/L) ³	16.28 ± 1.19 ^c	49.72 ± 3.58 ^a	28.43 ± 1.64 ^b	19.32 ± 1.67 ^c	15.09 ± 1.51 ^c	< 0.001
AST (U/L) ³	81.92 ± 5.19 ^d	189.8 ± 12.30 ^a	154.4 ± 8.20 ^b	106.6 ± 6.43 ^{cd}	118.6 ± 5.12 ^c	< 0.001
LDH (U/L) ³	709 ± 49.33 ^c	1502 ± 67.22 ^a	1362 ± 52.66 ^a	968 ± 45.93 ^b	854 ± 40.99 ^{bc}	< 0.001
Creatinine (mg/dL)	0.35 ± 0.05 ^c	1.02 ± 0.07 ^a	0.77 ± 0.08 ^b	0.39 ± 0.04 ^c	0.48 ± 0.06 ^c	< 0.001
Urea (mg/dL)	4.62 ± 0.78 ^c	13.63 ± 1.27 ^a	9.68 ± 0.68 ^b	7.44 ± 0.84 ^{bc}	5.74 ± 0.77 ^c	< 0.01
Uric acid (mg/dL)	2.04 ± 0.27 ^c	5.38 ± 0.63 ^a	4.46 ± 0.60 ^{ab}	2.96 ± 0.41 ^{bc}	2.46 ± 0.36 ^c	< 0.01

Means in the same raw with no superscript letters after them or with a common superscript letter following them are not significantly different (P < 0.05).

^a SE: standard error.

^b AG: Albumin: globulin ratio, ALT: alanine amino transferase, AST: aspartate amino transferase, LDH: Lactate dehydrogenase.

3.5. Lipid profile

The impact of supplementing YSE at concentrations of 6, 8, and 10 mg/L on the lipid profile of *O. niloticus* in the presence of NH₃ pollution (5 mg/L) is summarized in Table 6. Also in this case, a significant increase in all parameters of group 2 with respect to group 1 ($P < 0.001$) was observed. All parameters showed a significant mitigating effect of the group 3, 4 and 5 ($P < 0.001$) with respect to group 2, except for HDL, with group 4 and 5 being able to completely restore the baseline levels or improve them.

3.6. Immunity and antioxidants

The influences of supplementing with YSE at 6, 8, and 10 mg/L water every two days against NH₃ pollution (5 mg/L) on the immune system and antioxidant profiles of *O. niloticus* are illustrated in Table 7. The findings revealed a significant decrease of all parameters in group 2 with respect to group 1 ($P < 0.001$) with the only exception of MDA, which was significantly increased ($P < 0.001$). Moreover, groups 3, 4 and 5 of C3, IgA, lysozyme, SOD, CAT, GSH and TAC showed a significant improvement in a dose-dependent manner with respect to group 2 ($P < 0.001$), except for group 3 of IgA, significantly exceeding the baseline values in group 5 ($P < 0.001$), except for group 5 of SOD ($P < 0.05$). Although IgG and IgM values showed a similar trend, a significant increase with respect to those of the baseline was only observed in groups 4 and 5 ($P < 0.001$ for IgG and $P < 0.05$ for IgM). As expected, MDA values significantly increased in group 2 with respect to group 1 ($P < 0.001$), while reached significantly lower values in groups 3, 4 and 5 with respect to groups 1 and 2 ($P < 0.001$).

3.7. Digestive enzymes

The impact of supplementing YSE (at concentrations of 6, 8, and 10 mg/L of water every two days) on the digestive enzymes of *O. niloticus* in the presence of NH₃ pollution (5 mg/L) is summarized in Table 8. Lipase and amylase showed two different patterns. The first was significantly reduced in groups 2, 3 and 5 ($P < 0.001$) with respect to group 1, while it was significantly increased in group 4 ($P < 0.001$). As for the second, groups 2, 3, and 4 resulted significantly reduced with respect to group 1, while group 4 resulted significantly increased ($P < 0.001$).

4. Discussion

Fish reproduction, growth, and ability to withstand stressful situations are all impacted by the excessive accumulation of inorganic nitrogen compounds, especially ammonia, from organic material, fish feces, and residual feed (Romano, Zeng, 2013). These consequences may lower fish reproduction, feeding behavior, survival, and performance, resulting in a loss of fish productivity and significant financial losses (Ip et al., 2010; Shahzad et al., 2022). The principal strategies and measures to mitigate ammonia toxicity include ceasing feeding, modifying the

water, increasing the dissolved oxygen levels, employing microorganisms particular to nitrification, or cautiously adding lime to the ponds (Dawood et al., 2019). Furthermore, yucca extract application is potent for lowering the NH₃ concentration in fish ponds (Yang et al., 2015). Our finding revealed a significant improvement in water quality by YSE supplementation. These results agree with the following researchers who stated that adding YSE, the rearing water quality of mirror carp (Wang et al., 2020), juvenile European seabass (Elkhatay et al., 2019), striped catfish (Guroy et al., 2014), and Nile tilapia (Abdel-Tawwab et al., 2021) was improved, and the accumulated ammonia levels decreased. The decrease in NH₃ concentration is ascribed to the interaction of NH₃ combined with steroidal saponins and glycols, or the transformation of NH₃ into nitrite and nitrate, and the diminished concentration of NH₃ is associated with reduced water temperature and pH (Abdel-Tawwab et al., 2021).

Our results demonstrated a significant enhancement in growth performance by YSE supplementation in the water of Nile tilapia. These results align with Abdel-Tawwab et al. (2021) in Nile tilapia treated with YSE and/or *Saccharomyces cerevisiae* yeast added to water at a concentration of 1 g/m³ for 8 weeks. The water additives enhanced the Nile tilapia growth results ($P < 0.05$), with the fish treated with YSE + yeast exhibiting the maximum growth. Similarly, for 45 days, Fayed et al. (2019) investigated the effects of 3 YSE levels (0.25, 0.50, and 0.75 mg/L) in the water on young European seabass (5.0 g), growth performance improved ($P < 0.05$) when YSE concentration in the water elevated in concert with improvements in water quality, and this research aligns with our findings. El-Saidy and Gaber (2004) also demonstrated the impact of graded *Y. schidigera* powder supplementation (0, 250, 500, 750, 1000, 1250, or 1500 mg/kg diet) on *O. niloticus* and the feeding trials lasted 30-week and the findings demonstrated that FBW, WG, and SGR were boosted by treatment at dosages of 750 or 1000 mg/kg diet relative to the control group. The weight improvement and FCR reduction resulting from YSE treatment might be attributed to enhanced protein and amino acid synthesis, digestive enzyme activity, and improved absorption of nutrients.

Hematological parameters have been considered an important biomarker of fish intoxication. YSE supplementation in the water of Nile tilapia significantly affects hematological parameters such as Hb, RBCs, and WBCs, as well as glucose concentration in the blood. It also significantly alleviated oxidative stress by decreasing cortisol and nitric oxide blood levels. Increased blood ammonia could also induce the rupture of a high number of RBC, resulting in hemodilution (Abdo et al., 2022). It significantly decreased the concentration of RBC and Hb ($P < 0.05$). This effect was documented in several fish species (Abdo et al., 2022; Uchenna et al., 2022). This finding aligns with Reham et al. (2019), who reported analogous results for the hematology of Nile tilapia (51 g starting weight) after a 93-day administration of YSE in water.

Furthermore, Fayed et al. (2019) reported that the inclusion of YSE in aquatic environments enhances the hematological profile and immune response of juvenile European seabass, evidenced by increased counts of WBCs, RBCs, hematocrit (Hct), hemoglobin (Hb), lymphocytes, and neutrophils, mainly at a concentration of YSE (0.75 mg/L).

Table 6

Effect of *Yucca schidigera* extract (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on lipid profile ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters ^b	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
TC (mg/dL)	125.41 ± 6.10 ^c	181.75 ± 5.26 ^a	145.64 ± 6.91 ^b	127.95 ± 4.92 ^c	122.94 ± 4.37 ^c	< 0.001
TG (mg/dL)	281.48 ± 5.89 ^c	371.85 ± 10.84 ^a	310.39 ± 12.43 ^{bc}	315.48 ± 9.80 ^b	283.65 ± 8.77 ^{bc}	< 0.01
HDL (mg/dL)	42.82 ± 4.47 ^{ab}	28.19 ± 1.77 ^c	29.24 ± 1.14 ^c	35.78 ± 1.14 ^{bc}	45.35 ± 1.89 ^a	< 0.01
LDL (mg/dL)	26.30 ± 3.63 ^c	79.18 ± 4.65 ^a	54.32 ± 4.18 ^b	29.07 ± 2.39 ^c	20.86 ± 3.64 ^c	< 0.001
VLDL (mg/dL)	56.30 ± 1.18 ^c	74.37 ± 2.17 ^a	62.08 ± 2.49 ^{bc}	63.10 ± 1.96 ^b	56.73 ± 1.75 ^{bc}	< 0.001
LDL/HDL	0.62 ± 0.08 ^c	2.83 ± 0.26 ^a	1.85 ± 0.07 ^b	0.81 ± 0.06 ^c	0.46 ± 0.07 ^c	< 0.001

Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

^a SEM e standard error.

^b TC: total cholesterol, TG: triglycerides, HDL: high density lipoprotein, LDL: low density lipoprotein and VLDL: Very low-density lipoprotein.

Table 7

Effect of *Yucca schidigera* extract (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on immunity and antioxidants ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters ^b	Group 1	Group 2	Group 3	Group 4	Group 5	P-value
C ₃ (mg/dL)	9.85 ± 1.05 ^a	3.82 ± 0.70 ^c	5.63 ± 0.72 ^{bc}	8.17 ± 0.93 ^{ab}	11.06 ± 1.08 ^a	< 0.01
IgM (ng/ml)	0.66 ± 0.05 ^a	0.37 ± 0.05 ^c	0.48 ± 0.02 ^b	0.69 ± 0.02 ^a	0.69 ± 0.02 ^a	< 0.001
IgG (ng/ml)	0.49 ± 0.05 ^{bc}	0.35 ± 0.02 ^d	0.43 ± 0.03 ^{cd}	0.68 ± 0.04 ^a	0.61 ± 0.05 ^{ab}	< 0.01
IgA (ng/ml)	0.85 ± 0.04 ^{ab}	0.53 ± 0.04 ^c	0.55 ± 0.05 ^c	0.78 ± 0.03 ^b	0.91 ± 0.02 ^a	< 0.001
Lysozyme (ng/ml)	0.52 ± 0.05 ^a	0.36 ± 0.04 ^b	0.47 ± 0.05 ^{ab}	0.51 ± 0.05 ^a	0.60 ± 0.05 ^a	< 0.05
MDA (nmol/ml)	1.09 ± 0.05 ^b	1.32 ± 0.07 ^a	0.91 ± 0.04 ^{bc}	0.95 ± 0.09 ^{bc}	0.83 ± 0.05 ^c	< 0.01
SOD (U/ml)	0.41 ± 0.04 ^a	0.23 ± 0.02 ^b	0.32 ± 0.03 ^{ab}	0.46 ± 0.05 ^a	0.44 ± 0.05 ^a	< 0.05
CAT (ng/ml)	0.68 ± 0.04 ^{ab}	0.44 ± 0.03 ^c	0.57 ± 0.04 ^b	0.71 ± 0.04 ^a	0.77 ± 0.03 ^a	< 0.001
GSH (mmol/ml)	0.73 ± 0.04 ^a	0.44 ± 0.04 ^b	0.48 ± 0.05 ^b	0.66 ± 0.03 ^a	0.79 ± 0.04 ^a	< 0.001
TAC (ng/ml)	0.62 ± 0.05 ^{ab}	0.41 ± 0.04 ^c	0.54 ± 0.05 ^{bc}	0.65 ± 0.04 ^{ab}	0.71 ± 0.04 ^a	< 0.01

Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

^a SE: standard error.

^b C₃: complement 3, IgM: immunoglobulin M, IgG: immunoglobulin G, IgA: immunoglobulin A, MDA: Malondialdehyde, SOD: Superoxide dismutase, CAT: Catalase, GSH: Reduced glutathione and TAC: Total antioxidant capacity.

Table 8

Effect of *Yucca schidigera* extract (YSE) supplementation (6, 8 and 10 mg/L water every two days) against ammonia pollution (5 mg/L) on digestive enzymes ($\bar{X} \pm SE^a$) of *O. niloticus*.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	P value ²
Lipase (U/L)	41.06 ± 2.27 ^{ab}	25.15 ± 2.00 ^d	30.72 ± 1.97 ^{cd}	46.38 ± 2.88 ^a	37.82 ± 3.22 ^{bc}	< .01
Amylase (U/L)	65.78 ± 2.58 ^a	53.24 ± 2.02 ^b	54.21 ± 2.18 ^b	63.38 ± 1.90 ^a	66.81 ± 1.73 ^a	< 0.01

Means in the same row with no superscript letters after them or with a common superscript letter following them are not significantly different ($P < 0.05$).

^a SE: standard error.

The treatment of ammonia toxicity using YSE significantly modulated the ammonia effect as they restored the hematological indices, WBC, and Hb to their normal levels compared to the non-stressed group. Our findings agree with [Elbially et al. \(2021\)](#), who stated that YSE and BS improved hematological parameters against different types of stressors in Nile tilapia.

YSE supplementation to the water of Nile tilapia significantly improved liver and kidney function tests by increasing total protein, albumin, and globulin and reducing AST, ALT, LDH, creatinine, urea, and uric acid. Our findings align with [Abdel-Tawwab et al. \(2021\)](#), who documented decreased levels of AST, uric acid, and creatinine following YSE and/or the yeast *Saccharomyces cerevisiae* application as a water supplement. Furthermore, YSE supplementation in the water of Nile tilapia significantly improved lipid profile by reducing TC, TG, LDL, and VLDL and elevating HDL blood levels. Our findings are matched with the results of [Abdel-Tawwab et al. \(2021\)](#), who documented enhancements in the blood profile of Nile tilapia, characterized by elevated serum total lipids, total protein, albumin, and globulin, alongside reduced concentrations of aspartate aminotransferase, uric acid, and creatinine, following using yeast *S. cerevisiae* or YSE as a water supplementation method. YSE supplementation to the Nile tilapia water significantly improved the immunological status in Nile tilapia by increasing complement 3, lysozyme levels, IgM, IgG, and IgA in the Nile tilapia blood. Our outcomes align with [Cheek et al. \(2006\)](#), who demonstrated that *Yucca* has a high concentration of bioactive substances, such as alkaloids, terpenoids, saponins, steroids, phenolics, tannins, glycosides, and flavonoids, which are thought to be the reason for its effectiveness as a natural immunostimulant. Moreover, [Wang et al. \(2020\)](#) findings in this regard suggest that dietary YSE incorporation may enhance the immunity of mirror carp through an increase in lysozyme activity (LYZ) when YSE is included at 200 and 400 mg/kg diet, as well as an increase in C3 and C4. When given *yucca* as food, the aquatic animals demonstrated directly increased local intestinal immunity, which was associated with

an improvement in the immune system overall because of the impact of *yucca* on pathogenic microorganisms as an antibacterial agent in the digestive system (GIT) ([Dawood et al., 2021](#)). *Yucca* reduces gastrointestinal irritation caused by disease and toxins and reduces stress caused by adverse aquaculture conditions ([Njagi et al., 2017](#)).

Furthermore, antioxidant activity such as SOD, CAT, GSH, and TAC of *O. niloticus* was significantly improved by YSE supplementation in the water of the Nile tilapia. These outcomes agree with the outcomes of [Wang et al. \(2020\)](#), who documented that *Yucca* therapy significantly produced an active antioxidative capacity and an increased immunological response. Moreover, [Abdel-Tawwab et al. \(2021\)](#) observed increased SOD, CAT, and GPx activities along with a decreased MDA value when YSE + yeast (*S. cerevisiae*) water additions were added at 1.0 g/L Nile tilapia in ($p < 0.05$). Similarly, [Bae et al. \(2020\)](#) stated that using olive flounder with *Yucca* meal at 1.5 mg/kg diet enhanced SOD compared to the control group. Medical plants and their products are thought to possess different functions as antioxidants and immunostimulants ([Stratev et al., 2018](#); [Mehrinakhi et al., 2020](#)). *Yucca* herb and its derivatives have shown antioxidant activities attributed to its phenolic hydroxyl groups (hydrogen donors), which lowers the formation of hydroxyl peroxide ([Mohammadi et al., 2020](#)).

Furthermore, digestive enzymes, especially lipase and amylase, were significantly improved by YSE supplementation in the water of the Nile tilapia. These results agree with those of [Hernández-Acosta et al. \(2016\)](#), who demonstrated that the improvement in weights and reduction in FCR of white shrimp resulting from *Y. schidigera* and *Quillaja saponaria* treatments might be attributed to enhanced digestive enzyme activity and improved nutrition absorption. Using *yucca* products has a favorable impact on the growth rate, feed conversion ratio, and health of fish ([Wang et al., 2020](#)). The enhancement in growth and other health indices as a result of *yucca* supplements may be linked to the improvement in water quality and feed utilization, which relies on the intestinal status by modulating the enzyme activity, gut microbiota, and absorption ([Gaber et al., 2006](#); [Elkhatay et al., 2019](#)). In the same context, [Abdo et al. \(2022\)](#) suggested that the *Bacillus* strains probiotic mixture (BS)-YSE mix could treat aquatic ammonia and alleviate ammonia toxicity in fish.

5. Conclusion

According to the findings of our study, the effectiveness of YSE in mitigating the negative impact of ammonia intoxication was attributed to its powerful antioxidant activities and immunostimulant characteristics. In Nile tilapia, the findings of this research indicated that YSE treatment was helpful for both the growth and health of the fish and that YSE might be utilized as a practical water additive in aquaculture in the future.

Author statement

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Declaration of Competing Interest

There were no conflict of interests.

Acknowledgment

This work was supported by the Researchers Supporting Project (RSPD2025R731), King Saud University (Riyadh, Saudi Arabia).

Data availability

The data that has been used is confidential.

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