Aquaculture 534 (2021) 736287

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

The regulatory roles of yucca extract on the growth rate, hepato-renal function, histopathological alterations, and immune-related genes in common carp exposed with acute ammonia stress

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ARTICLE INFO

Keywords:
Ammonia
Yucca schidigera
Carp
Water quality
Health condition

ABSTRACT

The accumulation of ammonia in fish ponds regularly occurs in intensive aquaculture systems, and the inclusion of yucca extract is recognized as a practical solution to adsorb the waterborne ammonia. In this context, the study was planned to investigate the possible regulatory roles of yucca extract on the performances of common carp exposed to acute ammonia stress. Fish with similar initial weight were assigned to four groups (triplicates) where fish in the control group reared without ammonia exposure and without yucca treatment whereas the second group was exposed to ammonia (10 mg/L) without yucca treatment. The third group treated with yucca (0.75 mg/L) without ammonia while the fourth group exposed with ammonia and treated with yucca. The groups were named as the control, the ammonia, the yucca, and the ammonia/yucca. After 30 days, the growth performance, survival rate, total protein, albumin, and globulin of fish treated with yucca extract had the highest values ($P < 0.05$) followed by control and those exposed to acute ammonia stress and treated with yucca. The feed conversion ratio (FCR) displayed the lowest value in fish treated with yucca without ammonia stress while the highest FCR was in fish exposed to ammonia without yucca ($P < 0.05$). The uric acid and urea levels displayed the lowest value in fish treated with yucca without ammonia stress while the highest uric acid and urea were in fish exposed to ammonia without yucca ($P < 0.05$). The levels of blood creatinine, aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) implemented the highest values in fish exposed to acute ammonia stress without yucca while the lowest values were in the group of fish treated with yucca without ammonia exposure ($P < 0.05$). The expression of hepatic catalase (CAT), superoxide dismutase (SOD), and interleukin (IL-10) genes were upregulated in fish treated with yucca and downregulated in fish exposed to acute ammonia stress. Heat shock protein (HSP70), interleukin (IL-8), tumor necrosis factor-alpha (TNF-α), interferon gamma (IFN-γ), and interleukin (IL-1β) were upregulated in fish exposed to acute ammonia stress and downregulated in the group of fish treated with yucca extract ($P < 0.05$). The histopathological study revealed that fish exposed to acute ammonia stress had inflammatory and abnormal features while yucca extract induced anti-inflammation influences. Hence, the study concluded that the treatment of yucca extract is recommended to protect common carp from the toxicity of waterborne ammonia.

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https://doi.org/10.1016/j.aquaculture.2020.736287
Received 15 July 2020; Received in revised form 7 November 2020; Accepted 15 December 2020
Available online 17 December 2020

1. Introduction

Aquaculture is one of the fastest-growing industries in food production and plays a vital role in meeting the needs of dietary protein for humans (FAO, 2020; Tacon, 2020). Correspondingly, intensive fish farming is recognized as the most reasonable aquaculture system, which depends on growing of certain species of fish in the available amount of water (Badiola et al., 2018; Dawood, 2020). Notably, intensive systems cause the accumulation of ammonia that results from the feces of fish, the remaining amounts of feed, the increased sedimentation of organic matter in the bottom of ponds, and the lack of dissolved oxygen (Sriyasak et al., 2015). Concurrently, the fluctuations between the water acidity (pH) and temperature affect the ratio of the formed unionized ammonia (NH_3) that causes toxicity and harmful impacts on the ecosystem and aquatic organisms (Mook et al., 2012; Taheri Mirghaed et al., 2019; Yousefi et al., 2020). Severe ammonia toxicity induces several effects on the aquatic animals, including the decrease of feed consumption, deteriorated physiological functions, unstable breathing through gills, oxidative stress, diminished immunity, and inflammatory features in the gills (Cheng et al., 2015; Qi et al., 2017; Yue et al., 2010). The metabolism of protein inside the body is also can be deteriorated, which consumes high amounts of energy to balance the protein level in the fish body (Güroy et al., 2014). The main solutions to lower the ammonia toxicity is to stop feeding, change the water, increase the level of dissolved oxygen, use nitrification specific bacteria, or carefully including lime in the ponds (Boyed, 1998). Additionally, the application of yucca extract is also recognized as a powerful tool to reduce the ammonia level in aquaculture ponds (Fayed et al., 2019; Yang et al., 2015).

Yucca schidigera is originally cultivated in Mexico and southwestern USA under the conditions of high temperature and lack of water (Tenon et al., 2017). Yucca has abundant amounts of polyphenolics, steroidal saponins, and resveratrol and can be used as a solution or as a powder (Adegbeye et al., 2019). Yucca is applied mainly to reduce the levels of ammonia emissions in aquaculture ponds due to its content of steroidal saponin fractions, which has surface-active properties and can bind to ammonia via glycol-component fractions (Cheeke, 2000; Yang et al., 2015). The reduced levels of accumulated ammonia would result in the balance of protein metabolism in the fish body and reduce energy consumption (Güroy et al., 2014). Hence, the feed utilization, growth performance, and physiological status of aquatic species can be improved by yucca. Additionally, the yucca application resulted in the enhancement of the antioxidative, immunological, and anti-inflammatory responses in several aquatic animals (Güroy et al., 2014; Yang et al., 2015; Fayed et al., 2019; Wang et al., 2020). In this sense, yucca is an alternative approach to overcome the excessive use of antibiotics for eco-friendly aquaculture.

Common carp (*Cyprinus carpio*) usually is cultured under stressful conditions associated with intensive aquaculture systems (Dawood and Koshio, 2016). More specifically, the intensive conditions elucidated abundant amounts of ammonia emissions, which induces oxidative stress, immunosuppression, and inflammatory features in the carp's body (Wang et al., 2020). Exclusively, the present study proposed a protective solution through the application of yucca extract in the rearing water of common carp exposed to ammonia accumulation. The study presented how can yucca protects against ammonia exposure by detecting the growth and survival rates, blood biochemical and immunological indices, the expressions of antioxidative and inflammatory genes, as well as the histopathological alterations of gills, intestines, and livers.

2. Materials and methods

2.1. Design and procedure

The experiment was performed by following the ethical guidelines of the Faculty of Agriculture, Kafrelsheikh University, Egypt. Common carp juveniles were gently transported from a local farm (Kafrelsheikh city) and kept in 500 L tanks for adaptation before the trial for 2 weeks. Fish were fed with the basal diet (30% crude protein, AQUA International for Food Industries, Cairo) 2 times daily during the acclimation period and the general health condition and movement were checked visually. Afterwards, fish were randomly distributed into four groups of glass aquaria (60 L) where each group contains 3 aquaria. Each aquarium was supplied with continuous aeration, labelled, and housed with 10 equal sized carps with an average initial weight of 25.46 ± 0.22 g. For 30 days, half of the water in each aquarium was replaced with newly dechlorinated water.

The four groups were named as the control group (without ammonia), ammonia group (Am, 10 mg/L), yucca group (0.75 mg/L), and ammonia/yucca group (10 mg/L ammonia+0.75 mg/L yucca). Yucca extract (Khirate El-Nile Company- Egypt) was supplied every 72 h to each aquarium by following Fayed et al. (2019). The solution of ammonia was prepared by using NH_4CL (100 g/L) and supplied to each aquarium at the rate of 10 mg/L. The level of ammonia in the rearing water was decided based on the findings of Qi et al. (2017) who concluded that 10 mg ammonia/L induce moderate impacts on the crucian carp (*Carassius auratus*) performances whereas the high level of ammonia toxicity severely impaired the immune and antioxidative responses. Fish in all groups fed the basal diet (30% crude protein) which prepared by following Dawood et al. (2020a) at the rate of 3% of the body weight two times daily (8:00 am and 3:00 pm). The feces of fish and the remaining amounts of feed were removed after 30 min of feeding to avoid the increased sedimentation of organic matter that can produce ammonia. The water characteristics were checked regularly during the trial by using a temperature, digital oxygen (DO), and pH meters (HANNA Instruments, US). Total ammonia levels were measured with by HACH comparison apparatus using HACK kits (Hach Co., Loveland, Colorado, USA). while un-ionized ammonia (NH_3) levels were calculated based on the water temperature and pH by following Emerson et al. (1975) and APHA (2005). The average water temperature, DO, pH, total ammonia, and NH_3 were tabulated in Table 2.

2.2. Final sampling

At the end of the trial, fish were fasted for 24 h before sampling and anesthetized by 100 mg Tricaine Methanesulfonate (MS222)/L. All fish were individually counted and weighed to calculate the growth performances using the following equations:

$$\begin{aligned}\text{Weight gain (WG, \%)} &= 100 \times (\text{final body weight (FBW, } g\text{)–initial body weight (IBW, } g\text{)})/\text{IBW (g)}; \text{Specific growth rate (SGR, \%/day)} \\ &= 100 \times (ln\ \text{FBW}–ln\ \text{IBW})/\text{days}; \text{Feed conversion ratio (FCR)} = \text{total dry feed intake (g)}/(\text{FBW}–\text{IBW}); \text{Survival (\%)} \\ &= 100 \times \text{final fish number}/\text{initial fish number}.\end{aligned}$$

Then, three fish per aquarium were bled from the caudal vein to collect the blood in Eppendorf tubes, and the collected blood was kept for 2 h to clot. The serum was collected after centrifugation at 3500 ×*g*

for 15 min at 4 °C and maintained at −20 °C for analysis. The serum related analysis was done after 1 week to avoid samples spoilage. The fish were dissected to obtain the gills, intestines, and livers tissues (40% ethyl alcohol) for the histopathological study. Meantime, a piece of the liver (100 mg) was immediately frozen in the liquid nitrogen for RNA extraction.

2.3. Serum biochemistry

Serum total proteins and albumins were determined, according to Doumas et al. (1981) and Dumas and Biggs (1972) while globulin content was calculated mathematically. Activities of aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferase (ALT) were determined calorimetrically at the wavelength 540 nm (Reitman and Frankel, 1957). Serum creatinine, uric acid, urea, and bilirubin were calorimetrically determined according to Heinegård and Tiderström (1973) and Coulombe and Favreau (1963), respectively.

2.4. Histopathology

The histopathological examination was adopted according to Gewaily et al. (2020). The samples of gills, intestines, and livers which were cut into pieces of approximately 0.5 cm^3 and fixed in Bouin's solution for 18–24 h. Then, the fixed samples were dehydrated in ascending grades of alcohol (70%, 80%, 90%, absolute I, II and absolute III), cleared with xylene, and embedded in paraffin wax. Then 5 μm thick sections were obtained with Leica rotatory microtome (RM 20352035; Leica Microsystems, Wetzlar, Germany) and stained with hematoxylin and eosin. Finally, the tissue sections were examined with a BX50/BXFLA microscope (Olympus, Tokyo, Japan).

2.5. Gene transcription

Total RNA was extracted from 50 mg of liver tissue using Trizol (iNtRON Biotechnology, Inc., Korea) according to the manufacturer's manual. After confirmation of the extracted RNA quality and quantity by Nanodrop (Uv-Vis spectrophotometer Q5000/ Quawell, USA). Complementary DNA (cDNA) was synthesized using SensiFAST™ cDNA synthesis kit (Bioline, United Kingdom) according to the manufacturer's protocol. Gene-specific primer sequences were used for HSP70, CAT, SOD, IL-1β, IL8, IL10, TNF-α, IFN-γ genes and β-actin (as a housekeeping gene) by following Ghelichpour et al. (2019) (Table 1). The quantitative real-time PCR (Stratagene MX3000P) was used for gene expression. SYBR green method was used to quantify the gene expression using RT-PCR (SensiFast SYBR Lo-Rox kit, Bioline). After verification of PCR efficiency to be around 100%, the gene expression data were calculated according to the method of Livak and Schmittgen (2001).

Table 1
Primers used for qRT-PCR analysis.

Gene	Primer	Accession no.
HSP70	F: TGTGAGCGAGCCAAGAGAACCC	XM_019074376.1
	R: AAGCGAGCTCTGGTGATGGACG	
SOD	F: TGAGCTGTCGGAAGCCATCAAG	XM_019111527.1
	R: TTGGTTCCCACATGCAGCAATCC	
CAT	R: AAGGTCCCAGTTGCCCTCATCG	GQ376154.1
	F: AGACGACACCCATCGCTGTTCG	
IL-1β	F: ACCAGCTGGATTTGTCAGAAG	AB010701.1
	R: ACATACTGAATTGAACTTTG	
IL-8	F: GTCTTAGAGGACTGGGTGTA	EU011243.1
	R: ACAGTGTGAGCTTGGAGGGA	
IL-10	F: CGCCAGCATAAAGAACTCGT	JX524550.1
	R: TGCCAAATACTGCTCGATGT	
IFN-γ	F: TCTTGAGGAACCTGAGCAGAA	NM_001361222.1
	R: TGTGCAAGTCTTTCCTTTGTAG	
TNF-α	F: GGTGATGGTGTCGAGGAGGAA	AJ311800.1
	R: TGGAAAGACACCTGGCTGTA	
β-Actin	F: CCTGTATGCCAACACCGTGCTG	JQ619774.1
	R: CTTCATGGTGGAGGGAGCAAGG	

HSP70: heat shock protein 70, CAT: catalase, SOD: superoxide dismutase, IL-1β: interleukin 1β, IL-8: interleukin 8, IL-10: interleukin 10, IFN-γ: interferon gamma, TNF-α: tumor necrosis factor-alpha, and: β-actin: house-keeping gene.

2.6. Statistical analysis

Normality and homoscedasticity analyses were established before applying a one-way ANOVA method to confirm the normal distribution of the data. Data were presented as means ± the standard error (SE) and were analyzed by one-way ANOVA method by using SPSS 22.0 (SPSS version 22, SPSS Inc., Il, USA). And then Duncan's multiple-range test was used to determine significances among treatments at $P < 0.05$.

3. Results

3.1. Water characteristics

No significant differences were observed among the groups in terms of the water temperature, pH, and dissolved oxygen on day 0 and after 15 or 30 days ($P > 0.05$) (Table 2). The values of total ammonia nitrogen (TAN) and un-ionized ammonia (NH_3) showed the highest levels in the group exposed to acute ammonia while the lowest levels were in the group of fish treated with yucca on day 0 and after 15 or 30 days ($P < 0.05$). The group of fish exposed to ammonia and treated with yucca displayed lower TAN and NH_3 than the group of fish exposed to ammonia without yucca ($P < 0.05$).

Table 2
Water characteristics measured during the trial.

		Ctrl	Am	Yucca	Am/ yucca	*P* value
Dissolved oxygen (mg/L)	0 day	6.02 ± 0.13	6.13 ± 0.16	6.48 ± 0.25	6.26 ± 0.06	0.061
	15 days	6.17 ± 0.09	6.25 ± 0.61	6.84 ± 0.49	6.34 ± 0.02	0.311
	30 days	6.22 ± 0.2	6.28 ± 0.13	6.31 ± 0.41	6.35 ± 0.22	0.062
Temperature (°C)	0 day	26.11 ± 0.15	26.13 ± 0.18	26.32 ± 0.2	26.26 ± 0.34	0.511
	15 days	25.05 ± 0.24	25.24 ± 0.16	25.11 ± 0.11	25.09 ± 0.26	0.426
	30 days	25.22 ± 0.52	25.33 ± 0.34	25.34 ± 0.08	25.26 ± 0.25	0.055
pH	0 day	7.23 ± 0.12	7.25 ± 0.11	7.13 ± 0.24	7.21 ± 0.05	0.223
	15 days	7.11 ± 0.15	7.25 ± 0.31	7.19 ± 0.01	7.24 ± 0.22	0.471
	30 days	7.32 ± 0.05	7.45 ± 0.03	7.21 ± 0.11	7.44 ± 0.15	0.231
TAN (mg/L)	0 day	1.08 ± 0.14^{c}	10.22 ± 0.32^{a}	0.85 ± 0.12^{d}	2.96 ± 0.15^{b}	0.002
	15 days	1.13 ± 0.11^{c}	10.53 ± 0.23^{a}	0.78 ± 0.01^{d}	3.21 ± 0.22^{b}	0.012
	30 days	1.02 ± 0.02^{c}	10.02 ± 0.01^{a}	0.82 ± 0.03^{d}	3.06 ± 0.02^{b}	0.005
NH_3-N (mg/L)	0 day	0.015 ± 0.001^{c}	0.151 ± 0.001^{a}	0.013 ± 0.002^{d}	0.042 ± 0.001^{b}	0.001
	15 days	0.016 ± 0.002^{c}	0.162 ± 0.002^{a}	0.014 ± 0.001^{d}	0.045 ± 0.002^{b}	0.021
	30 days	0.014 ± 0.001^{c}	0.141 ± 0.001^{a}	0.012 ± 0.002^{d}	0.043 ± 0.001^{b}	0.042

*TAN: total ammonia nitrogen, NH_3-N: un-ionized ammonia. Values with different letters in each row are significantly different from those of control group ($P < 0.05$).

Table 3
Growth performance of common carp reared in water with yucca and ammonia exposure for 30 days.

	Ctrl	Am	Yucca	Am/yucca	*P* value
IBW (g)	25.48 ± 0.20	25.42 ± 0.21	25.49 ± 0.20	25.51 ± 0.24	0.211
FBW (g)	36.98 ± 0.80^{b}	33.52 ± 0.54^{c}	39.66 ± 0.87^{a}	37.23 ± 0.59^{b}	0.002
WG (%)	45.19 ± 4.07^{b}	31.85 ± 1.45^{c}	55.62 ± 3.97^{a}	45.93 ± 1.87^{b}	0.023
SGR (%/day)	1.24 ± 0.09^{a}	0.92 ± 0.04^{b}	1.47 ± 0.08^{a}	1.26 ± 0.04^{a}	0.004
FCR	2.15 ± 0.13^{b}	2.57 ± 0.05^{a}	1.67 ± 0.07^{c}	1.90 ± 0.09^{bc}	0.001
Survival (%)	96.67 ± 3.33^{a}	85.00 ± 2.89^{b}	98.33 ± 1.67^{a}	95.00 ± 2.89^{a}	0.022

*Values expressed as means ± SE ($n = 3$). Values with different letters in each row are significantly different from those of control group ($P < 0.05$).

3.2. Growth performance

The results illuminated that the final body weight (FBW) and weight gain (WG) of the group of fish treated with yucca extract had the highest values ($P < 0.05$) followed by control and those exposed to acute ammonia stress and treated with yucca. Nonetheless, the lowest FBW and WG was in the group of fish exposed to acute ammonia stress for 30 days ($P < 0.05$) (Table 3). Notably, the specific growth rate (SGR) and survival rate were significantly higher in the control, yucca, and ammonia/yucca groups than the group of fish exposed to acute ammonia stress ($P < 0.05$) (Table 3). However, no significant differences were observed on SGR and survival rate of fish in the control, yucca, and ammonia/yucca groups ($P > 0.05$). The feed conversion ratio (FCR) displayed the lowest value in fish treated with yucca without ammonia stress while the highest FCR was in fish exposed to ammonia without yucca ($P < 0.05$) (Table 3). No significant differences were observed in terms of FCR between fish reared in the control and those exposed to ammonia and treated with yucca ($P > 0.05$).

Table 4
Blood biochemical parameters of common carp reared in water with yucca and ammonia exposure for 30 days.

	Ctrl	Am	Yucca	Am/yucca	*P* value
Uric acid (mg/dl)	1.91 ± 0.01^{b}	2.25 ± 0.08^{a}	1.56 ± 0.04^{c}	1.72 ± 0.03^{bc}	0.023
Creatinine (mg/dl)	0.24 ± 0.03^{b}	0.42 ± 0.05^{a}	0.21 ± 0.01^{b}	0.26 ± 0.01^{b}	0.001
Urea (mg/dl)	2.85 ± 0.16^{c}	4.39 ± 0.09^{a}	3.49 ± 0.08^{b}	3.13 ± 0.05^{bc}	0.022
Bilirubin (mg/dl)	3.48 ± 0.20^{a}	4.03 ± 0.12^{a}	2.60 ± 0.17^{b}	3.51 ± 0.13^{a}	0.031
Globulin (g/dl)	1.90 ± 0.08^{b}	1.64 ± 0.15^{b}	2.59 ± 0.15^{a}	1.97 ± 0.04^{b}	0.034
Albumin (g/dl)	1.50 ± 0.08^{b}	1.27 ± 0.05^{c}	1.84 ± 0.04^{a}	1.77 ± 0.07^{a}	0.002
Total protein (g/dl)	3.40 ± 0.16^{bc}	2.91 ± 0.18^{c}	4.43 ± 0.18^{a}	3.73 ± 0.05^{b}	0.042
ALP (U/l)	84.28 ± 3.08^{b}	92.96 ± 0.29^{a}	70.97 ± 0.50^{c}	80.88 ± 2.25^{b}	0.001
AST (U/l)	72.61 ± 0.85^{b}	83.86 ± 0.82^{a}	61.12 ± 0.75^{c}	64.58 ± 1.69^{c}	0.041
ALT (U/l)	3.21 ± 0.07^{b}	3.79 ± 0.09^{a}	2.72 ± 0.06^{c}	3.13 ± 0.05^{b}	0.002

*Values expressed as means ± SE ($n = 3$). Values with different letters in each row are significantly different from those of control group ($P < 0.05$).
Alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

3.3. Blood biochemistry

The uric acid and urea levels displayed the lowest value in fish treated with yucca without ammonia stress while the highest uric acid and urea were in fish exposed to ammonia without yucca ($P < 0.05$) (Table 4). No significant differences were observed in terms of uric acid and urea between fish reared in the control and those exposed to ammonia and treated with yucca ($P > 0.05$).

The levels of blood creatinine, ALT, AST, and ALP implemented the highest values in fish exposed to acute ammonia stress without the treatment of yucca ($P < 0.05$) (Table 4). Conversely, the lowest ALT, AST, and ALP were in the group of fish treated with yucca without ammonia exposure ($P < 0.05$) (Table 4). The creatinine displayed non-significant differences between the control, yucca, and yucca/ammonia groups ($P > 0.05$). The levels of ALP and ALT showed no significant differences between the control and yucca/ammonia groups whereas the groups of yucca and yucca/ammonia showed no differences in case of AST.

The highest total protein, albumin, and globulin were in fish treated with yucca only while the lowest values were in fish exposed to acute ammonia stress ($P < 0.05$) (Table 4). The levels of total protein and globulin showed no significant differences between the control and yucca/ammonia groups whereas the groups of yucca and yucca/ammonia showed no differences in case of albumin ($P > 0.05$).

3.4. Antioxidative and stress related genes

The mRNA levels of hepatic SOD and CAT genes were upregulated in fish treated with yucca and downregulated in fish exposed to acute ammonia stress compared to fish in the control and ammonia/yucca groups (Fig. 1A and B). The control and the group of fish exposed to acute ammonia and treated with yucca showed no significant differences ($P > 0.05$).

The expression of hepatic HSP70 gene was upregulated in fish exposed to acute ammonia stress without yucca and downregulated in fish treated with yucca (Fig. 1C). The control and the group of fish exposed to acute ammonia and treated with yucca showed no significant differences ($P > 0.05$).

3.5. Inflammatory related genes

The mRNA levels of pro-inflammatory genes (IL-8, TNF-α, IFN-γ, and IL-1β) were upregulated in fish exposed to acute ammonia stress and downregulated in the group of fish treated with yucca extract (Fig. 2A, B, C, and D) ($P < 0.05$). The control and the group of fish exposed to acute ammonia and treated with yucca showed no significant differences in terms of IL-8, IFN-γ, and IL-1β ($P > 0.05$). The expression of TNF-α gene in the group of fish exposed to ammonia and treated with yucca was higher than that in the control group ($P < 0.05$).

The expression of anti-inflammatory gene (IL-10) was upregulated in fish exposed to acute ammonia stress and treated with yucca but downregulated in the group of fish exposed to acute ammonia without the treatment of yucca extract (Fig. 2E) ($P < 0.05$). The control and the group of fish treated with yucca showed no significant differences in terms of IL-10 ($P > 0.05$).

3.6. Histopathology

The histological structure of gills in the control group revealed intact epithelium of both primary and secondary filaments (Fig. 3A). After exposure to ammonia (Fig. 3B), the gills showed degeneration, highly congested blood vessels in the primary and secondary epithelium as well as infiltration of inflammatory cells. In the group treated by yucca (Fig. 3C), the gills showed normal structure like the control group. In the group treated with yucca with ammonia (Fig. 3D), the epithelium appeared normal with mononuclear cells infiltration and slight vascular

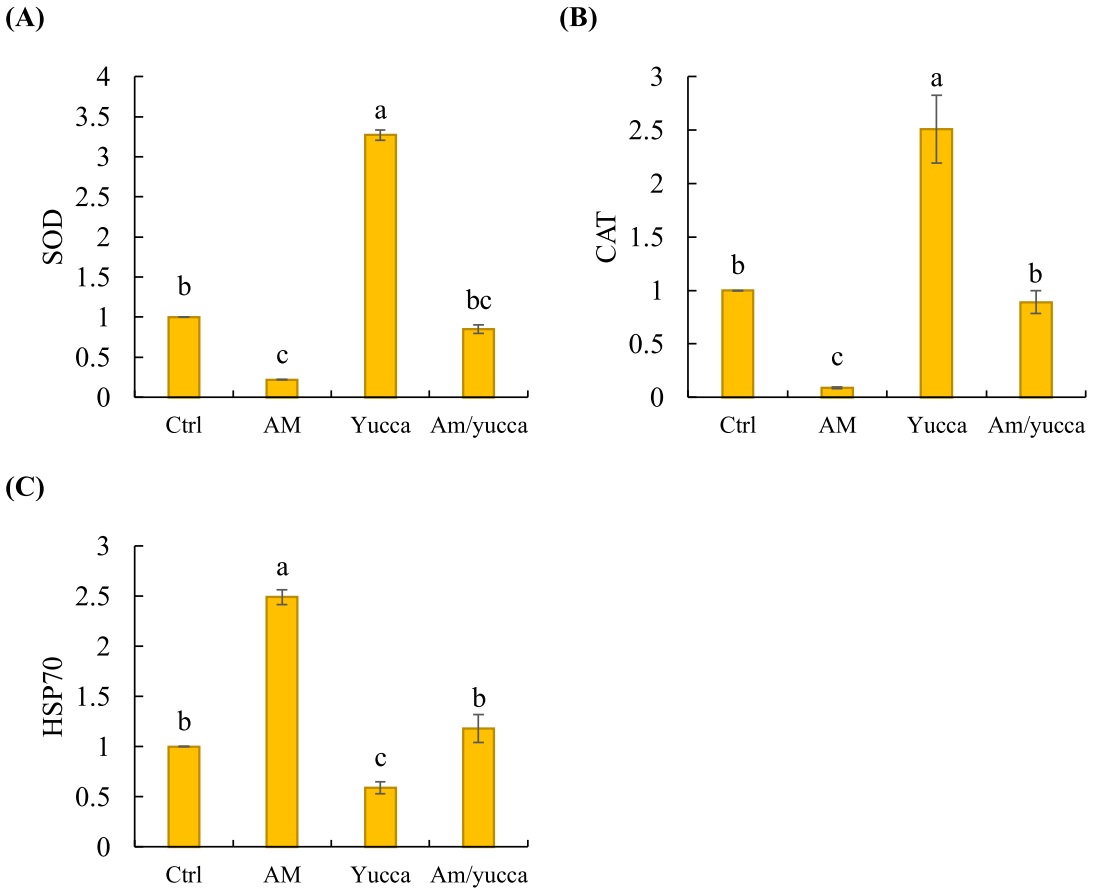

Fig. 1. Fold change in mRNA expression levels of antioxidative (SOD and CAT) and heat shock protein 70 (HSP70) genes of common carp reared in water with yucca and ammonia exposure for 30 days. Values are expressed as mean ± SE from triplicate groups. Bars with different letters are significantly different from those of control group ($P < 0.05$).

congestion.

The intestine in the control group had normal intestinal villi which lined by intact enterocytes with goblet cells (Fig. 4A). After exposure to ammonia, the intestine showed degeneration of the intestinal villi and submucosa with desquamation of the enterocytes (Fig. 4B). In the yucca group (Fig. 4C), the intestinal epithelium was healthy intact, and the intestinal villi were characterized by increased height and branching. In the group of both yucca and ammonia, the villi were normal and higher than the control group (Fig. 4D).

The histological structure of hepatopancrease was similar in the control and yucca groups (Fig. 5A and C). The hepatic part showed intact polyhedral cells arranged in cords separated by blood sinusoids. The cells of pancreatic acini appeared normal around hepatic vessels. On the other side, ammonia caused fatty degeneration of hepatocytes, congestion of blood sinusoids and degeneration of pancreatic cells (Fig. 5B). Pyknosis of hepatic cells was clearly found with presence of inflammatory cells around the degenerated pancreatic cells. The hepatopancrease revealed normal structure in case of adding yucca with ammonia (Fig. 5D) where, there was no degeneration of cells or congestion of vessels.

4. Discussion

The waterborne ammonia is a severe concern that weakens the performances and health status of aquatic animals (Cheng et al., 2015; Qi et al., 2017; Yue et al., 2010). The use of yucca extract is an essential approach that effectively reduces the undesirable effects of high ammonia level in fish farming (Adegbeye et al., 2019).

The results displayed reduced levels of accumulated ammonia and NH_3 in the group of fish exposed to ammonia and treated with yucca extract for 30 days. In a similar sense, the inclusion of yucca extract mediated the quality of rearing water and lowered the accumulated ammonia in case of mirror carp (Wang et al., 2020), Nile tilapia (*Oreochromis niloticus*) (Abdel-Tawwab et al., 2020; Engler et al., 2018), striped catfish (*Pangasianodon hypophthalmus*) (Güroy et al., 2014), and European seabass juveniles (*Dicentrarchus labrax*) (Fayed et al., 2019). Yucca extract has steroidal saponins and glycol with active surface attributed to ammonia's adsorption (Cheeke, 2000; Piacente et al., 2005). Correspondingly, the reduction in ammonia levels is probably attributed to the binding of ammonia with steroidal saponins and glycols or the transformation of ammonia to nitrite and nitrate (Abdel-Tawwab et al., 2020; Headon and Dawson, 1990). Furthermore, the decreased level of NH_3 is correlated with the decreased level of pH and water temperature (Abdel-Tawwab et al., 2020). In this context, Fayed et al. (2019) elucidated that yucca extract reduced the level of pH in water. Concurrently, the level of NH_3 is probably lowered due to the relative reduction in the level of pH in rearing water.

The growth performance of carps treated with yucca either with or without ammonia exposure displayed increased FBW, WG, and SGR rates. The improved growth performance is probably attributed to yucca extract's role in enhancing the palatability of diets by activating the digestive enzymes. More specifically, the steroidal saponins helps in increasing the absorption of nutrients by increasing the permeability of intestinal barriers (Francis et al., 2002; Yang et al., 2015). Concurrent with these results, in the yucca group, the intestinal epithelium was healthy intact, and the intestinal villi were characterized by increased height and branching. Also, yucca extract can improve the intestinal wall integrity by increasing the intestinal mucosa's thickness and

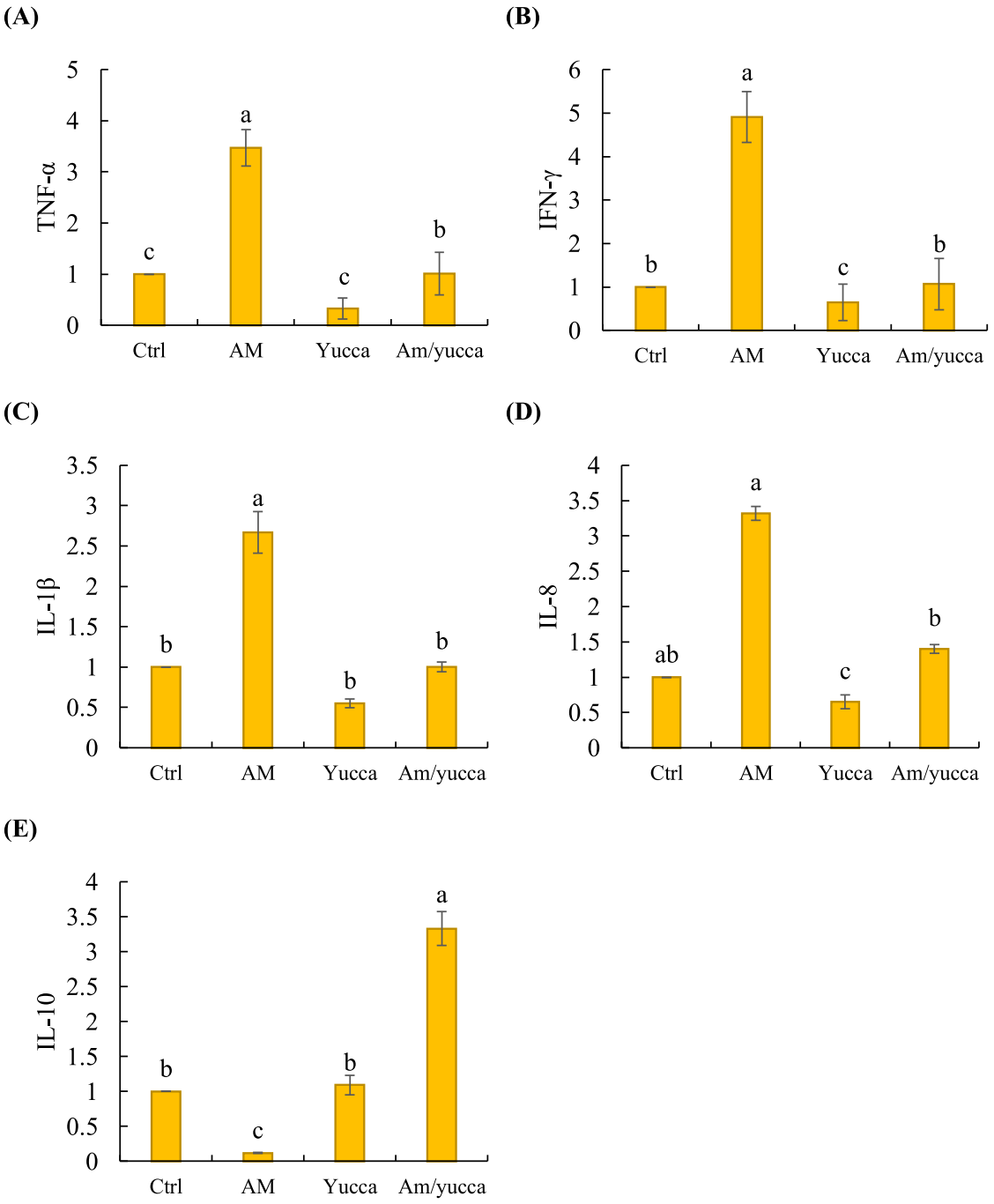

Fig. 2. Fold change in mRNA expression levels of inflammatory genes of common carp reared in water with yucca and ammonia exposure for 30 days. Values are expressed as mean ± SE from triplicate groups. Bars with different letters are significantly different from those of control group ($P < 0.05$).

increasing bacterial growth (Huang et al., 2005). In this regard, the results exhibited enhanced feed efficiency (reduced FCR), which indicates the efficient digestibility of diets in fish treated with yucca. The improved feed efficiency is associated with yucca's role in increasing the viability of intestinal microbiota that can secret digestive enzymes to facilitate the absorption of nutrients (Wang et al., 2020). Omnivorous fish species (including common carp) have filter-feeding habits, which means that fish can graze and ingest nutrients and substances available in the rearing water, including phytoplankton and yucca extract (Rahman et al., 2006). Therefore, yucca extract in rearing water is expected to improve growth rate and feed efficiency in common carp.

Notably, the survival rate of fish exposed to ammonia is declined, but those treated with yucca exhibited enhanced survival rates. The lowered survival rate is attributed to the deteriorated health condition induced by continuous ammonia stress for 30 days (Rajabiesterabadi et al., 2020). Conversely, the treatment with yucca resulted in high survival rates indicating enhanced immunity and well-being of common carp.

The detection of biochemical indices is associated with hepatic, renal, anemic, and immune functions, as well as the level of lipid and protein metabolism in the blood of the organism (Burgos-Aceves et al., 2019; Dawood et al., 2020c). Correspondingly, the general physiological and health condition of fish reared under stressful conditions or fed-specific feed formulations can be well characterized (Abbas, 2006; Rajabiesterabadi et al., 2020). The levels of blood protein (TP), albumin, and globulin were lowered in fish under ammonia stress, while fish treated with yucca revealed increased levels (Faggio et al., 2014). These

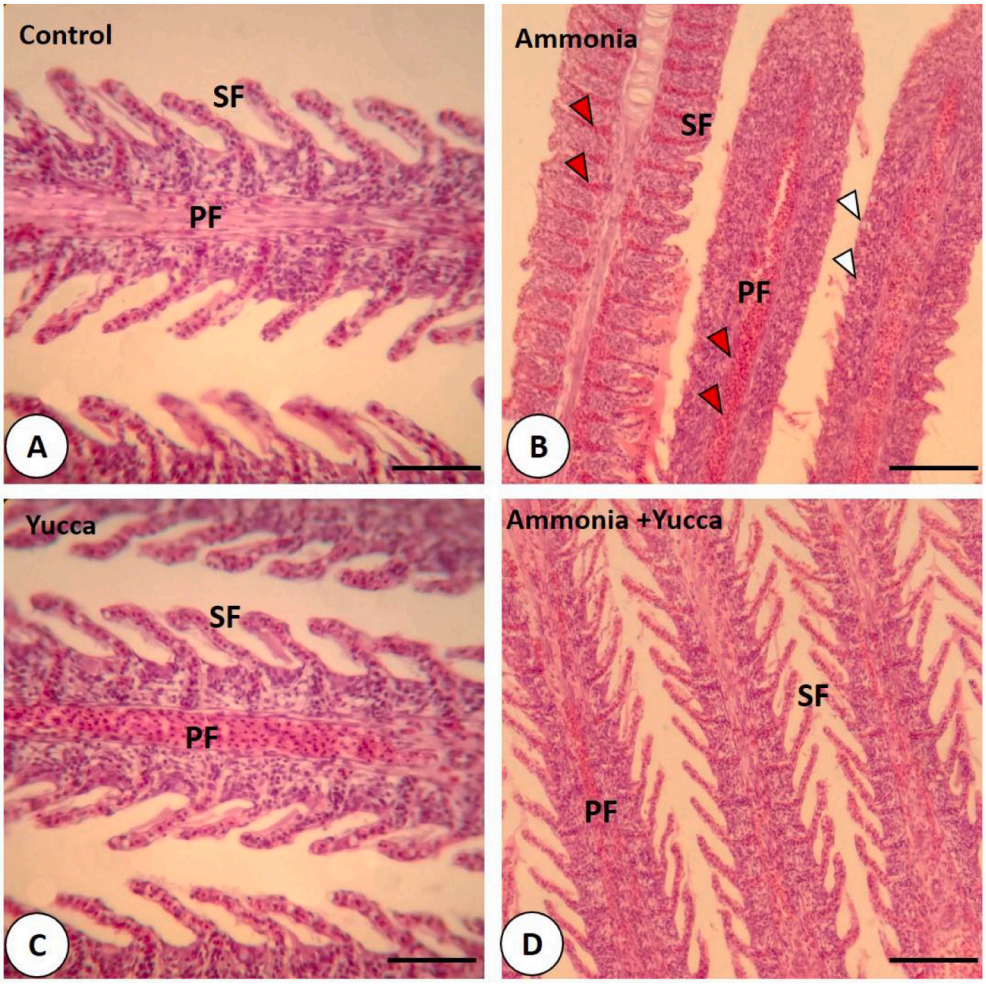

Fig. 3. Histomicrograph showing the histological structure of gills of common carp in the control group (A) as well as other treated groups by ammonia (B), Yucca (C) and Yucca with ammonia (D). In A and C, the gills show normal histological structures including intact primary (PF) and secondary (SF) filaments. The effect of ammonia (B) causes congestion of blood vessels of primary and secondary filaments (red arrow head) with accumulation of mononuclear cell around the respiratory epithelium of the secondary filaments (white arrow head). In group D, the gills show relatively normal structure with slight vascular congestion. Stain H&E. Bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

results refer to the severe impact of ammonia on the metabolism of proteins in the blood (Mustafa et al., 2014). It has been reported that blood proteins and globulins are associated with fish's general immune status and can be deteriorated under stressful conditions (Dawood et al., 2020c). The reduced TP, albumins, and globulin are also associated with the impaired immune system in common carp induced by ammonia exposure. Interestingly, yucca mediated TP, albumin, and globulin levels, which related to the role of yucca in regulating the metabolism of the nutrients in fish reared under optimal or stressful conditions (Adegbeye et al., 2019). Besides, the increased TP, albumin, and globulin levels are probably attributed to the stimulation of DNA, formation of ribosomes, and the proliferation of protein synthesis in the liver tissues, which led to the activated immune response in the fish body (Akrami et al., 2015; Mohammadi et al., 2020). Similarly, Abdel-Tawwab et al. (2020) stated that the inclusion of yucca in the ponds of Nile tilapia resulted in enhanced TP, albumin, and globulin levels. The level of uric acid and urea in the blood is the indicator of the level of the produced urea from the liver of fish (Kohn et al., 2005), and high levels of urea indicate the dysfunction of the renal tissue (Uchino et al., 2012). The results exhibited increased uric acid and urea in common carp reared under ammonia stress while fish treated with yucca displayed reduced levels. The reduced levels of urea in the blood can be explained by the role of steroidal saponin to inhibit ammonia production due to its surface-active properties and the binding of ammonia formation by glycol content (Adegbeye et al., 2019; Cheeke, 2000). In this sense, the results also showed increased ALT, ALP, and AST in the blood of fish exposed to ammonia stress. The increased ALT, ALP, and AST are attributed to ammonia's adverse impact on the liver function of fish (Abbas, 2006; Fayed et al., 2019). Further, the continuous stressful conditions altered the reactive oxygen species (ROS) in the liver of fish that induces oxidative stress and the damage of cell function (Dawood et al., 2020b; Lee et al., 2017). Nonetheless, yucca mediated the levels of ALT, ALP, and AST in fish due to the presence of saponin content that reduces the ammonia and urea emissions in the blood of fish (Piacente et al., 2005). It has also been reported that the active components of yucca (e.g., saponins) can regulate the serum enzyme activities correlated to the liver function in Nile tilapia (Abdel-Tawwab et al., 2020). In line with the present study, Fayed et al. (2019) reported that the levels of ALT and AST were reduced in seabass fed diets with yucca and stressed by ammonia exposure.

The detection of creatinine in the blood refers to the renal tissue's ability to regulate the creatinine content presented in the muscles of fish (Campbell, 2004). The results displayed increased creatinine levels in the blood of fish exposed to ammonia; however, fish treated with yucca showed low levels. These results indicate that common carp suffered from stressful conditions had damaged renal function. Concurrent with the present study, Abdel-Tawwab et al. (2020) reported that yucca treatment regulated creatinine levels in Nile tilapia and enhanced its resistance to ammonia accumulation. The role of yucca extract in regulating the level of urea and creatinine in the kidney is associated with abundant contents of saponin and stilbenes (Duffy et al., 2001).

Toxicity with ammonia usually induces adverse effects in the aquatic organisms, including oxidative stress and impaired immunity (Chen et al., 2020a; Qi et al., 2017; Yousefi et al., 2020; Zhang et al., 2020). The results exhibited downregulated SOD and CAT genes in fish exposed with ammonia. However, the inclusion of yucca in the rearing water-

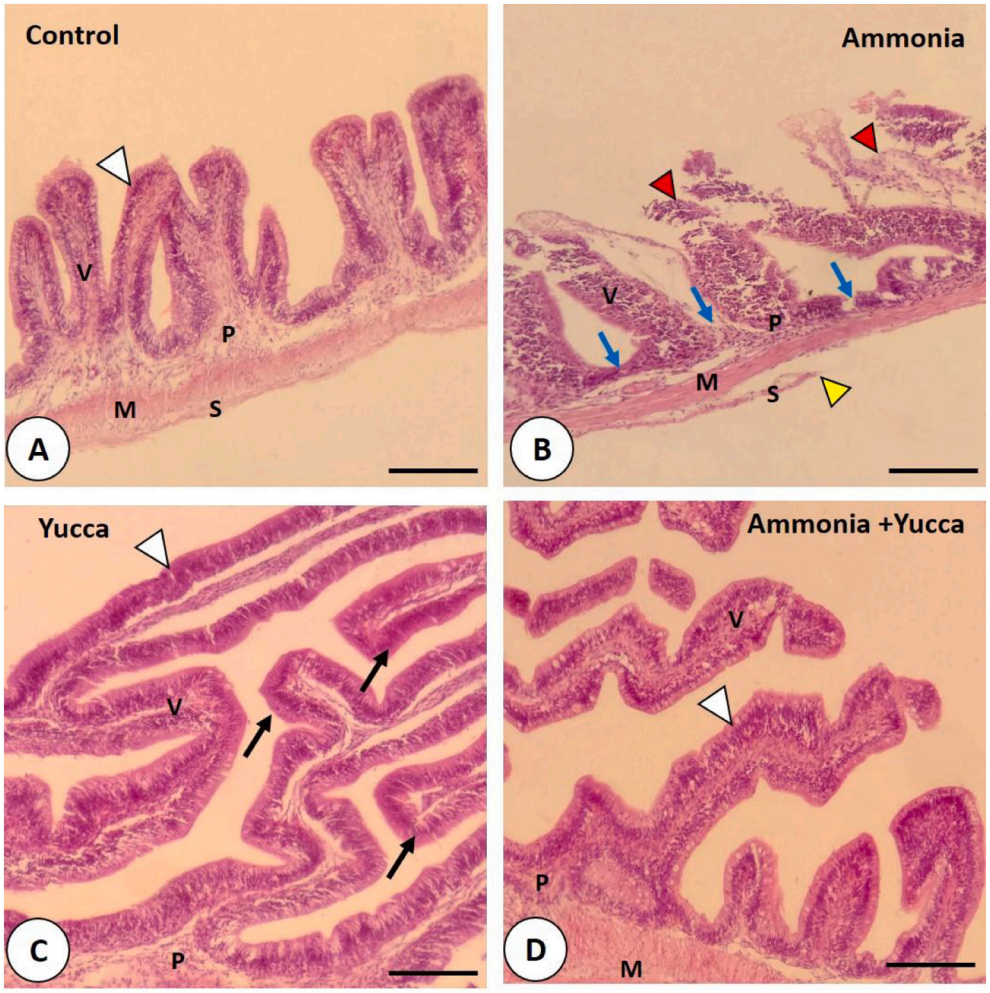

Fig. 4. Histomicrograph showing the histological structure of intestine of common carp in the control group (A) as well as other treated groups by ammonia (B), Yucca (C) and Yucca with ammonia (D). In group A, the intestine showed normal histological structures of the intestinal villi (V), lamina propria sub mucosa (P), tunica muscularis (M) and tunica serosa (S). In group C, there were increased height of intestinal villi which showed clear branching (C). The effect of ammonia (B) caused degeneration and separation of the intestinal villi epithelium (red arrow head) degeneration of crypts cells (blue arrow) and sloughing of intestinal serosa (yellow arrow head). In group D, the intestinal villi are intact and appeared normal like the control group but more crowded and higher. Stain H&E. Bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mediated the antioxidative response by upregulating the expression of SOD and CAT genes. Similarly, Wang et al. (2020), who reported that mirror carp fed yucca and exposed to ammonia toxicity showed improved antioxidative status. Yucca has abundant polyphenols that scavenge the overproduction of ROS in the cell (Hakkı Cigerci et al., 2009). These compounds also possess antioxidants and free-radical hunters that could aid in suppressing the reactive oxygen free-radicals (Ahmadifar et al., 2020). In this way, the yucca protects the cell's DNA damage by decreasing the lipid peroxidation (Martínez-Álvarez et al., 2005). Concurrent with the present study, Abdel-Tawwab et al. (2020) implemented that Nile tilapia treated with yucca and exposed to acute ammonia stress displayed reduced oxidative stress and lipid peroxidation.

Pro-inflammatory cytokines (IFN-γ, IL-8, TNF-α, and IL-1β) and stress (HSP70) related genes in the present study displayed upregulated mRNA levels in fish exposed with ammonia, but the treatment with yucca downregulated the (IFN-γ, IL-8, TNF-α, IL-1β, and HSP70) genes with or without ammonia stress. Conversely, the expression of ani-inflammatory (IL-10) gene was upregulated by the treatment of yucca while exposure with ammonia downregulated the expression of IL-10. In a similar sense, Wang et al. (2020) illustrated that the treatment with yucca induced upregulated IL-10 and downregulated IL-8, TNF-α, and IL-1β in mirror carp. The related inflammatory genes inhibit the inflammation features by enhancing immunity (Chen et al., 2020b; Ghelichpour et al., 2019; Jia et al., 2019; Jiaxin et al., 2020), while HSP70 is upregulated during stressful conditions to reduce the adverse impacts of stress on the fish body (Jun et al., 2015). The downregulated IFN-γ, IL-8, TNF-α, IL-1β, and HSP70 genes in fish treated with yucca indicates the balance between the inflammatory and anti-inflammatory responses attributed to the potent role of yucca as a natural immunostimulant and anti-inflammatory agent (Adegbeye et al., 2019). Attractively, saponins have been reported to induce the expression of cytokines and interferons through the production of complexes with immunostimulant properties (e.g., saponin, cholesterol, phospholipid, and amphipathic proteins) (Piacente et al., 2005). The present study displayed exclusive and exciting results about the potential impact of yucca extract to cope with the adverse effects of ammonia exposure through the regulation of IL-8, TNF-α, IFN-γ, IL-1β, and HSP70 genes in the liver of common carp.

Ammonia stress-induced the histopathological damage of livers, intestines, and gills of common carp. However, the groups of fish treated with yucca revealed normal histopathological features. The gills function in fish is to extract the ammonia from the water beside its role in the osmoregulation. The deterioration of gills in the control group refers to the damage of epithelium induced by high levels of ammonia (Abdel-Tawwab et al., 2020), but including yucca resulted in normal features. The high amount of ammonia in the rearing water causes impaired respiration function through the gills of fish (Sattari et al., 2013). Correspondingly, fish consume high amounts of accumulated energy to obtain their basic oxygen requirements (Scott and Rogers, 1980). The potential impact of yucca on the protection of livers and intestines from the harmful influences of ammonia is not well documented. However, the effect of yucca on the histopathological features of fish exposed to ammonia can be explained by the antioxidative and anti-inflammation

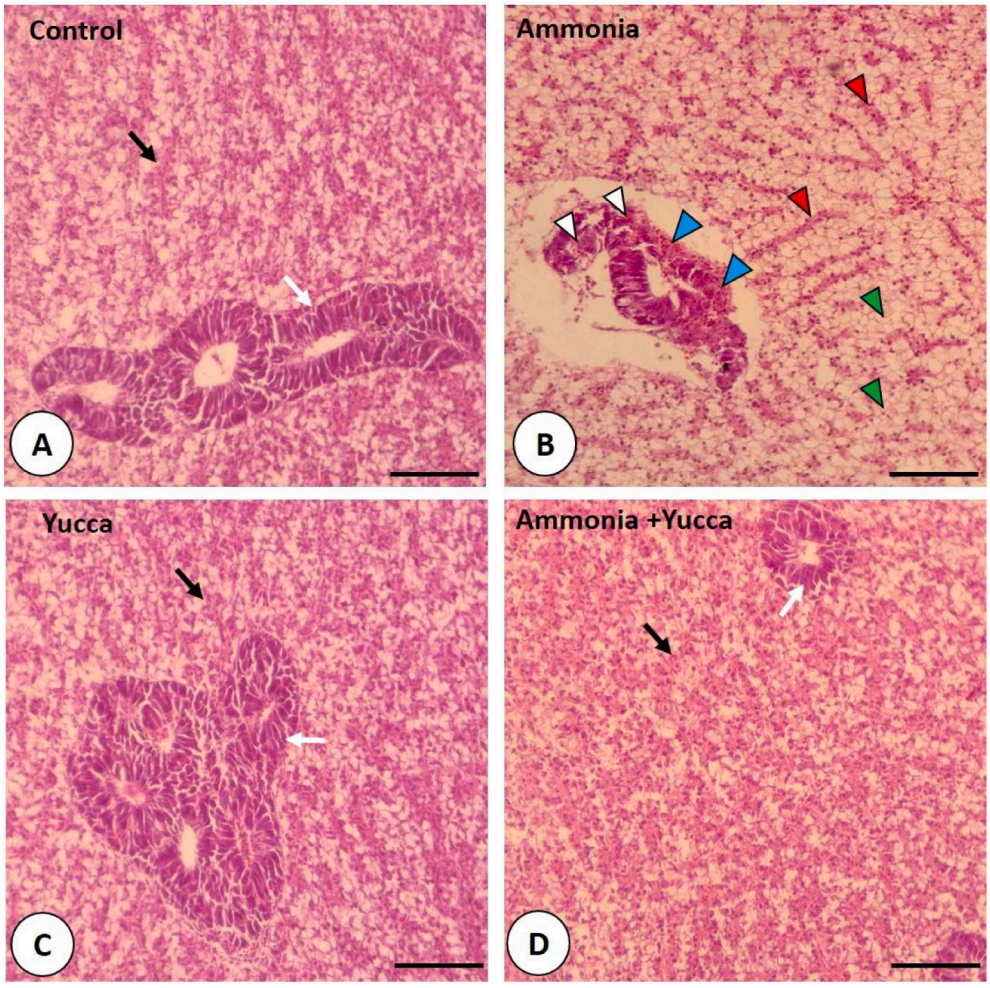

Fig. 5. Histomicrograph showing the histological structure of liver of common carp in the control group (A) as well as other treated groups by ammonia (B), Yucca (C) and Yucca with ammonia (D). In A and C, hepatopancrease shows normal polyhedral hepatocyte arranged in cords like appearance separated by blood sinusoids (black arrow) and pancreatic cells that form the pancreatic cover around hepatic vessels (white arrow). The effect of ammonia (B) causes fatty degeneration of hepatocytes (green arrow head), congestion of blood sinusoids (red arrow head), degeneration of pancreatic cells (white arrow head) and infiltration of mononuclear cells near the pancreatic acini (blue arrow head). In group D, the hepatopancrease retained its normal structure in its hepatic (black arrow) and pancreatic part (white arrow). Stain H&E. Bar = 100 μm. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

capacity. Yucca inhibited the inflammatory compromises through the mediation of the hepatic enzymes (ALT, ALP, and AST) and anti-oxidative response (SOD and CAT), as well as the regulation of pro-inflammatory (IFN-γ, IL-8, TNF-α, and IL-1β), anti-inflammatory (IL10), and stress (HSP70) related genes.

5. Conclusion

In conclusion, the study elucidated that common carp treated with yucca extract for 30 days had enhanced growth rate, antioxidative, and anti-inflammatory responses to counteract with the impacts of ammonia exposure. Yucca extract mediated the inflammation in gills, intestines, and livers tissues through the regulation of the antioxidative, anti-inflammatory, and stress-related genes. Additionally, yucca extracts alleviated the hepatic and renal alterations induced by ammonia exposure. Hence, the study concluded that the treatment of yucca extract is recommended to protect common carp from the toxicity of waterborne ammonia.

Declaration of Competing Interest

The authors declare no conflicts of interest.

Acknowledgment

This work was funded by Researchers Supporting Project (RSP-2020/36), King Saud University, Riyadh, Saudi Arabia. This research work was partially supported by Chiang Mai University.

References

Abbas, H.H., 2006. Acute toxicity of ammonia to common carp fingerlings (*Cyprinus carpio*) at different pH levels. Pak. J. Biol. Sci. 9, 2215–2221.

Abdel-Tawwab, M., Mounes, H.A.M., Shady, S.H.H., Ahmed, K.M., 2020. Effects of yucca, *Yucca schidigera*, extract and/or yeast, *Saccharomyces cerevisiae*, as water additives on growth, biochemical, and antioxidants/oxidant biomarkers of Nile tilapia, *Oreochromis niloticus*. Aquaculture 736122. https://doi.org/10.1016/j.aquaculture.2020.736122.

Adegbeye, M.J., Elghandour, M.M.M.Y., Monroy, J.C., Abegunde, T.O., Salem, A.Z.M., Barbabosa-Pliego, A., Faniyi, T.O., 2019. Potential influence of Yucca extract as feed additive on greenhouse gases emission for a cleaner livestock and aquaculture farming - a review. J. Clean. Prod. 239, 118074.

Ahmadifar, E., Yousefi, M., Karimi, M., Fadaei Raieni, R., Dadar, M., Yilmaz, S., Dawood, M.A.O., Abdel-Latif, H.M.R., 2020. Benefits of dietary polyphenols and polyphenol-rich additives to aquatic animal health: an overview. Rev. Fish. Sci. Aquacult. 1–34. https://doi.org/10.1080/23308249.2020.1818689.

Akrami, R., Gharaei, A., Mansour, M.R., Galeshi, A., 2015. Effects of dietary onion (*Allium cepa*) powder on growth, innate immune response and hemato–biochemical parameters of beluga (*Huso huso* Linnaeus, 1754) juvenile. Fish Shellfish Immunol. 45, 828–834.

APHA, 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington, DC, USA.

Badiola, M., Basurko, O.C., Piedrahita, R., Hundley, P., Mendiola, D., 2018. Energy use in recirculating aquaculture systems (RAS): a review. Aquac. Eng. 81, 57–70.

Boyed, C., 1998. Water quality for pond aquaculture. Res. Dev. Ser. 37.

Burgos-Aceves, M.A., Lionetti, L., Faggio, C., 2019. Multidisciplinary haematology as prognostic device in environmental and xenobiotic stress-induced response in fish. Sci. Total Environ. 670, 1170–1183.

Campbell, T., 2004. Clinical Chemistry of Fish and Amphibians. Veterinary Hematology and Clinical Chemistry. Lippincott Williams Wilkins, Pennsylvania, pp. 499–517.

Cheeke, P., 2000. Actual and Potential Applications of *Yucca schidigera* and *Quillaja saponaria* Saponins in Human and Animal Nutrition, Saponins in food, FEEDSTUFFS and Medicinal Plants. Springer, Dordrecht, pp. 241–254.

Chen, D., Ning, F., Zhang, J., Tang, Y., Teng, X., 2020a. NF-κB pathway took part in the development of apoptosis mediated by miR-15a and oxidative stress via

mitochondrial pathway in ammonia-treated chicken splenic lymphocytes. Sci. Total Environ. 729, 139017.
Chen, J., Zhang, S., Tong, J., Teng, X., Zhang, Z., Li, S., Teng, X., 2020b. Whole transcriptome-based miRNA-mRNA network analysis revealed the mechanism of inflammation-immunosuppressive damage caused by cadmium in common carp spleens. Sci. Total Environ. 717, 137081.
Cheng, C.-H., Yang, F.-F., Ling, R.-Z., Liao, S.-A., Miao, Y.-T., Ye, C.-X., Wang, A.-L., 2015. Effects of ammonia exposure on apoptosis, oxidative stress and immune response in pufferfish (*Takifugu obscurus*). Aquat. Toxicol. 164, 61–71.
Coulombe, J.J., Favreau, L., 1963. A new simple semimicro method for colorimetric determination of urea. Clin. Chem. 9, 102–108.
Dawood, M.A.O., 2020. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Rev. Aquacult. 13, 642–663.
Dawood, M.A.O., Koshio, S., 2016. Recent advances in the role of probiotics and prebiotics in carp aquaculture: a review. Aquaculture 454, 243–251.
Dawood, M.A., Eweedah, N.M., Moustafa, E.M., El-Sharawy, M.E., Soliman, A.A., Amer, A.A., Atia, M.H., 2020a. Copper nanoparticles mitigate the growth, immunity, and oxidation resistance in common carp (*Cyprinus carpio*). Biol. Trace Elem. Res. 1–10.
Dawood, M.A.O., El-Shamaa, I.S., Abdel-Razik, N.I., Elkomy, A.H., Gewaily, M.S., Abdo, S.E., Soliman, A.A., Paray, B.A., Abdelkhalek, N., 2020b. The effect of mannanoligosaccharide on the growth performance, histopathology, and the expression of immune and antioxidative related genes in Nile tilapia reared under chlorpyrifos ambient toxicity. Fish Shellfish Immunol. 103, 421–429.
Dawood, M.A.O., El-Salam Metwally, A., Elkomy, A.H., Gewaily, M.S., Abdo, S.E., Abdel-Razek, M.A.S., Soliman, A.A., Amer, A.A., Abdel-Razik, N.I., Abdel-Latif, H.M.R., Paray, B.A., 2020c. The impact of menthol essential oil against inflammation, immunosuppression, and histopathological alterations induced by chlorpyrifos in Nile tilapia. Fish Shellfish Immunol. 102, 316–325.
Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T., Schaffer, R., 1981. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin. Chem. 27, 1642–1650.
Duffy, C.F., Killeen, G.F., Connolly, C.D., Power, R.F., 2001. Effects of dietary supplementation with *Yucca schidigera* Roezl ex Ortgies andiIts saponin and non-saponin fractions on rat metabolism. J. Agric. Food Chem. 49, 3408–3413.
Dumas, B.T., Biggs, H.G., 1972. Standard Methods of Clinical Chemistry. Academic Press, New York.
Emerson, K., Russo, R.C., Lund, R.E., Thurston, R.V., 1975. Aqueous ammonia equilibrium calculations: effect of pH and temperature. J. Fish. Board Canada 32, 2379–2383.
Engler, P., Caillis, P., Voller, S., Labrie, L., 2018. Dietary supplementation of a mixture of saponin-rich plants to reduce ammonia-nitrogen excretion in juvenile Nile tilapia (*Oreochromis niloticus*). Sustain. Food Product. 6.
Faggio, C., Fedele, G., Arfuso, F., Panzera, M., Fazio, F., 2014. Haematological and biochemical response of *Mugil cephalus* after acclimation to captivity. Cah. Biol. Mar. 55, 31–36.
FAO, 2020. The State of World Fisheries and Aquaculture 2020. Sustainability in Action. Food and Agriculture Organization of the United Nations.
Fayed, W.M.A., Khalil, R.H., Sallam, G.R., Mansour, A.T., Elkhayat, B.K., Omar, E.A., 2019. Estimating the effective level of *Yucca schidigera* extract for improvement of the survival, haematological parameters, immunological responses and water quality of European seabass juveniles (*Dicentrarchus labrax*). Aquacult. Rep. 15, 100208.
Francis, G., Kerem, Z., Makkar, H.P., Becker, K., 2002. The biological action of saponins in animal systems: a review. Br. J. Nutr. 88, 587–605.
Gewaily, M.S., Kassab, M., Farrag, F.A., Almadaly, E.A., Atta, M.S., Abd-Elmaksoud, A., Wakayama, T., 2020. Comparative expression of cell adhesion molecule1 (CADM1) in the testes of experimental mice and some farm animals. Acta Histochem. 122, 151456.
Ghelichpour, M., Taheri Mirghaed, A., Hoseinifar, S.H., Khalili, M., Yousefi, M., Van Doan, H., Perez-Jimenez, A., 2019. Expression of immune, antioxidant and stress related genes in different organs of common carp exposed to indoxacarb. Aquat. Toxicol. 208, 208–216.
Güroy, B., Mantoğlu, S., Kayalı, S., Şahin, İ., 2014. Effect of dietary *Yucca schidigera* extract on growth, total ammonia–nitrogen excretion and haematological parameters of juvenile striped catfish *Pangasianodon hypophthalmus*. Aquac. Res. 45, 647–654.
Hakkı Cigerci, I., Fidan, A.F., Konuk, M., Yuksel, H., Kucukkurt, I., Eryavuz, A., Baysu Sozbilir, N., 2009. The protective potential of *Yucca schidigera* (Sarsaponin 30®) against nitrite-induced oxidative stress in rats. J. Nat. Med. 63, 311–317.
Headon, D., Dawson, K., 1990. Yucca extract controls atmospheric ammonia levels. Feedstuffs 62, 15–16.
Heinegård, D., Tiderström, G., 1973. Determination of serum creatinine by a direct colorimetric method. Clin. Chim. Acta 43, 305–310.
Huang, H., Zheng, G., Chen, F., 2005. *Yucca schidigera* planta natural multi-functional feed additives. Anim. Sci. Abroad 32, 61–64.
Jia, R., Li, Y., Cao, L., Du, J., Zheng, T., Qian, H., Gu, Z., Jeney, G., Xu, P., Yin, G., 2019. Antioxidative, anti-inflammatory and hepatoprotective effects of resveratrol on oxidative stress-induced liver damage in tilapia (*Oreochromis niloticus*). Comp. Biochem. Physiol. C 215, 56–66.
Jiaxin, S., Shengchen, W., Yirong, C., Shuting, W., Shu, L., 2020. Cadmium exposure induces apoptosis, inflammation and immunosuppression through CYPs activation and antioxidant dysfunction in common carp neutrophils. Fish Shellfish Immunol. 99, 284–290.
Jun, Q., Hong, Y., Hui, W., Didlyn, K.M., Jie, H., Pao, X., 2015. Physiological responses and HSP70 mRNA expression in GIFT tilapia juveniles, *Oreochromis niloticus* under short-term crowding. Aquacult. Res. 46, 335–345.
Kohn, R., Dinneen, M., Russek-Cohen, E., 2005. Using blood urea nitrogen to predict nitrogen excretion and efficiency of nitrogen utilization in cattle, sheep, goats, horses, pigs, and rats. J. Anim. Sci. 83, 879–889.
Lee, M., Lin, W., Yu, B., Lee, T., 2017. Antioxidant capacity of phytochemicals and their potential effects on oxidative status in animals—a review. Asian Austral. J. Anim. Sci. 30, 299.
Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta CT}$ method. Methods 25, 402–408.
Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defenses in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15, 75–88.
Mohammadi, G., Rafiee, G., El Basuini, M.F., Abdel-Latif, H.M.R., Dawood, M.A.O., 2020. The growth performance, antioxidant capacity, immunological responses, and the resistance against *Aeromonas hydrophila* in Nile tilapia (*Oreochromis niloticus*) fed Pistacia vera hulls derived polysaccharide. Fish Shellfish Immunol. 106, 36–43.
Mook, W.T., Chakrabarti, M.H., Aroua, M.K., Khan, G.M.A., Ali, B.S., Islam, M.S., Abu Hassan, M.A., 2012. Removal of total ammonia nitrogen (TAN), nitrate and total organic carbon (TOC) from aquaculture wastewater using electrochemical technology: a review. Desalination 285, 1–13.
Mustafa, S.A., Karieb, S.S., Davies, S.J., Jha, A.N., 2014. Assessment of oxidative damage to DNA, transcriptional expression of key genes, lipid peroxidation and histopathological changes in carp *Cyprinus carpio* L. following exposure to chronic hypoxic and subsequent recovery in normoxic conditions. Mutagenesis 30, 107–116.
Piacente, S., Pizza, C., Oleszek, W., 2005. Saponins and phenolics of *Yucca schidigera* Roezl: chemistry and bioactivity. Phytochem. Rev. 4, 177–190.
Qi, X.-Z., Xue, M.-Y., Yang, S.-B., Zha, J.-W., Wang, G.-X., Ling, F., 2017. Ammonia exposure alters the expression of immune-related and antioxidant enzymes-related genes and the gut microbial community of crucian carp (*Carassius auratus*). Fish Shellfish Immunol. 70, 485–492.
Rahman, M.M., Verdegem, M.C.J., Nagelkerke, L.A.J., Wahab, M.A., Milstein, A., Verreth, J.A.J., 2006. Growth, production and food preference of rohu *Labeo rohita* (H.) in monoculture and in polyculture with common carp *Cyprinus carpio* (L.) under fed and non-fed ponds. Aquaculture 257, 359–372.
Rajabiesterabadi, H., Yousefi, M., Hoseini, S.M., 2020. Enhanced haematological and immune responses in common carp *Cyprinus carpio* fed with olive leaf extract-supplemented diets and subjected to ambient ammonia. Aquac. Nutr. 26, 763–771.
Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63.
Sattari, M., Bagherzadeh Lakani, F., Sharifpour, I., Kazemi, R., 2013. Effect of hypoxia, normoxia and hyperoxia conditions on gill histopathology in two weight groups of beluga (*Huso huso*). Caspian J. Environ. Sci. 11, 77–84.
Scott, A.L., Rogers, W.A., 1980. Histological effects of prolonged sublethal hypoxia on channel catfish *Ictalurus punctatus* (Rafinesque). J. Fish Dis. 3, 305–316.
Sriyasak, P., Chitmanat, C., Whangchai, N., Promya, J., Lebel, L., 2015. Effect of water de-stratification on dissolved oxygen and ammonia in tilapia ponds in Northern Thailand. Int. Aquat. Res. 7, 287–299.
Tacon, A.G.J., 2020. Trends in global aquaculture and aquafeed production: 2000–2017. Rev. Fish. Sci. Aquacult. 28, 43–56.
Taheri Mirghaed, A., Fayaz, S., Hoseini, S.M., 2019. Effects of dietary 1,8-cineole supplementation on serum stress and antioxidant markers of common carp (*Cyprinus carpio*) acutely exposed to ambient ammonia. Aquaculture 509, 8–15.
Tenon, M., Feuillère, N., Roller, M., Birtić, S., 2017. Rapid, cost-effective and accurate quantification of *Yucca schidigera* Roezl. steroidal saponins using HPLC-ELSD method. Food Chem. 221, 1245–1252.
Uchino, S., Bellomo, R., Goldsmith, D., 2012. The meaning of the blood urea nitrogen/creatinine ratio in acute kidney injury. Clin. Kidney J. 5, 187–191.
Wang, L., Wu, D., Fan, Z., Li, H., Li, J., Zhang, Y., Xu, Q., Wang, G., Zhu, Z., 2020. Effect of *Yucca schidigera* extract on the growth performance, intestinal antioxidant status, immune response, and tight junctions of mirror carp (*Cyprinus carpio*). Fish Shellfish Immunol. 103, 211–219.
Yang, Q.-H., Tan, B.-P., Dong, X.-H., Chi, S.-Y., Liu, H.-Y., 2015. Effects of different levels of *Yucca schidigera* extract on the growth and nonspecific immunity of Pacific white shrimp (*Litopenaeus vannamei*) and on culture water quality. Aquaculture 439, 39–44.
Yousefi, M., Vatnikov, Y.A., Kulikov, E.V., Plushikov, V.G., Drukovsky, S.G., Hoseinifar, S.H., Van Doan, H., 2020. The protective effects of dietary garlic on common carp (*Cyprinus carpio*) exposed to ambient ammonia toxicity. Aquaculture 526, 735400.
Yue, F., Pan, L., Xie, P., Zheng, D., Li, J., 2010. Immune responses and expression of immune-related genes in swimming crab *Portunus trituberculatus* exposed to elevated ambient ammonia-N stress. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 157, 246–251.
Zhang, Y., Liu, Q., Yin, H., Li, S., 2020. Cadmium exposure induces pyroptosis of lymphocytes in carp pronephros and spleens by activating NLRP3. Ecotoxicol. Environ. Saf. 202, 110903.