Aquaculture International
https://doi.org/10.1007/s10499-024-01642-w

RESEARCH

Camel whey protein hydrolysate diet mitigates alkaline stress–induced biochemical disorders and restores the target of rapamycin, *MAPK* pathway, and autophagy-related gene expression in Nile tilapia

Rowida E. Ibrahim · Gehad E. Elshopakey · Mohamed Y. M. Aly · Abdelwahab A. Abdelwarith · Elsayed M. Younis · Yasmina M. Abd-Elhakim, et al. ***[full author details at the end of the article]***

Received: 12 May 2024 / Accepted: 31 July 2024

Abstract
Exposure to alkaline stress is common in aquatic practices. The present research investigated the detrimental impacts of rearing Nile tilapia under alkaline water stress by investigating the liver function indices and transcriptomic profile. A 30-day study was conducted on 160 fish (16.02 ± 0.14 g) split into four groups, each with four replicates. Group 1 (G1) and G2 were fed on a basal diet fortified with 0 and 75 g/kg diet camel whey protein hydrolysates (CWP) and reared in freshwater (carbonate alkalinity = 1.4 mmol/L, pH = 7.19). Moreover, G3 and G4 were fed on a basal diet enriched with 0 and 75 g/kg diet CWP and reared in alkaline water (carbonate alkalinity = 23.8 mmol/L; pH = 8.65). The outcomes revealed elevated lipid indices (triglycerides, cholesterol, low- and high-density lipoproteins) and liver enzymes (alanine and aspartate aminotransferase, alkaline phosphatase, and gamma-glutamyltransferase) with lowered protein indices (total protein, albumin, and globulin) in alkaline-reared fish. Moreover, oxidative stress was initiated through lowered antioxidant enzymes (superoxide dismutase, catalase, and reduced glutathione) and higher malondialdehyde in the alkaline-exposed fish. Alkaline stress induced the activation of the mechanistic target of rapamycin and *MAPK* pathway (mitogen-activated protein kinase, c-Jun NH terminal kinase, and *MAPK-1*) with down-regulation of the autophagy-related genes (*ATG-5*, *ATG-7*, and *ATG-13*) and cathepsin B expression. Feeding on a CWP-supplemented diet resulted in significant modulation of the lipid profile, liver enzyme activity, and improvement in protein indices and antioxidant enzyme activity. Furthermore, modulation of the transcriptomic profile of the hepatic tissue of the alkaline-exposed fish was noticed due to feeding on a CWP-supplemented diet. Overall, CWP dietary addition at a level of 75 g/kg diet can alleviate the alkaline stress exposure in Nile tilapia. These outcomes could contribute to understanding the physiological circumstances of Nile tilapia when reared in alkaline water as well as provide a novel dietary additive for mitigating the bad consequences due to alkaline-stress exposure.

Keywords Oreochromis niloticus · Oxidative stress · Liver enzymes · Protein hydrolysate · Gene expression

Handling Editor: Daniel Merrifield

Published online: 21 August 2024

Introduction

Aquaculture contributes significantly to the security of the world's food supply and gives humans vital nutritional support (Zhang et al. 2022). Nonetheless, aquaculture's growth has been severely hampered by a lack of water (Dawood et al. 2021). Alkaline land represents one-third of the world's total land area, is found in more than 100 countries, and is formed by prolonged evaporation, droughts, or little rain (Zhang et al. 2023a, 2023b). Therefore, creating alkaline water would be a useful strategy to advance the expansion of aquaculture (Ondrasek and Rengel 2021), and it is imperative to maintain the sustainable development of aquaculture (Zhao et al. 2020).

However, alkaline stress has been investigated to induce detrimental sequences on fish well-being and productivity (Liu et al. 2023; Fan et al. 2021). Alkalinity is the primary stress aquatic animals suffer, which impedes the growth of aquaculture (Song et al. 2021). Stress due to alkalinity raises the blood bicarbonate (HCO3–) concentration and throws off the equilibrium of ammonia (NH3) and ammonium ($NH4^+$) as a result of a hydrogen (H^+) deficit, which ultimately leads to ammonia toxicity (Yao et al. 2010). Alkalosis causes abnormal metabolism of amino acids, which primarily explains why aquatic animals in saline-alkaline water have poor protein utilization and slowed growth (Fan et al. 2021).

The liver is one of the largest and most researched internal organs in fish (Long et al. 2022). In addition to metabolizing and detoxifying numerous xenobiotics and excreting toxins, it can also break down and eliminate nutrients taken in by the digestive system (Roques et al. 2020). Additionally, it is a crucial tissue during times of environmental stress and contributes a crucial role in detoxification and metabolism (Roychowdhury et al. 2021). Alkaline stress badly affects the liver tissue through elevation of the serum acid phosphatase, alkaline phosphatase, and alanine transaminase activity and initiates inflammatory responses (Zhou et al. 2024).

Excellent biological values and functional characteristics of whey proteins include their ability to bind fatty acids, minerals, and vitamins, as well as their abundance of essential amino acids (Guo 2019). One potential source of nutritive proteins in large quantities is camel whey proteins. Enzymatic hydrolysis is a common technique to produce bioactive peptides from different sources of proteins (Osman et al. 2021b; Saad et al. 2020; El-Sanatawy et al. 2021). Hydrolyzed proteins found in camel milk can produce a mixture of bioactive peptides. These peptides have functional characteristics, such as antioxidant, anti-hypertensive, antidiabetic, and antimicrobial properties (Abdel-Hamid et al. 2016, 2020; Osman et al. 2021a). These protein fractions are α-lactalbumin (α-LA) (>50%), immunoglobulins, lactoferrin, and camel serum albumin (CSA) (Momen et al. 2019). Bioactive peptides found in camel whey protein hydrolysates (CWP) have anti-inflammatory and antioxidant characteristics (Behrouz et al. 2022). CWP reduced heat stress–induced liver damage when tested in normal *Rattus norvegicus* hepatocyte cell culture by a concentration of 10, 30, and 50 μg/mL (Du et al. 2021) and prevented heat stress–related oxidative damage, inflammatory processes, and lymphocyte apoptosis in mice orally administered CWP at a dose of 100 mg/kg body weight (Badr et al. 2018).

Because of their high nutritional value and benefits to the economy, tilapias are widely used aquaculture species and are farmed in 127 countries (Wu et al. 2023). The main tilapia species raised for food is the Nile tilapia (*Oreochromis niloticus*), due to its capacity to withstand stress (Dawood et al. 2020).

This paper aims to utilize Nile tilapia as a valuable model species for examining the impact of alkalinity stress on various physiological aspects, including hepatic functioning enzymes, lipid metabolism, oxidative stress, inflammatory conditions, and the expression of genes associated with autophagy and inflammasome pathways. Additionally, we will explore the tissue microarchitecture alterations induced by alkalinity stress

on hepatic tissue. Furthermore, we seek to investigate the potential of dietary CWP in enhancing tolerance to alkalinity stress. Specifically, our focus will be on assessing the effects of CWP diets on hepatic function and structure.

Materials and methods

CWP preparation

Camel milk samples were obtained from a modern farm in Bilbies City (Sharkia Governorate, Egypt). We added 1 M HCl at pH 4.6 to precipitate the caseins (CNs) and then centrifuged (Jouan C4-22 Benchtop Centrifuge, France) the mixture (6000 × g, 1 h at 4 °C). After the whey was obtained, it was lyophilized, dialyzed against a 50 mM phosphate buffer (pH 7.8), and refrigerated until required. Enzymatic hydrolysis of camel whey protein was conducted using trypsin from porcine pancreas (EC3.4.21.4) (Merck, KGaA, Darmstadt, Germany). Under the following conditions, buffer, 0.1 M Na_2HPO_4-NaH_2PO_4; pH, 8.8; temperature, 37 °C; enzyme/substrate ratio, 1/200 E/S (w/w), we fully mixed the substrate and enzyme and then incubated them for 4 h at 37 °C with constant stirring. To inactivate the enzyme, we boiled the mixture in a bath for 10 min at 100 °C. Next, we centrifuged the CWP for 10 min at 5000 × g and 4 °C. Finally, the supernatant was lyophilized and stored at − 20 °C until use (Abdel-Hamid et al. 2017). The trichloroacetic acid (TCA) method was used to determine the degree of hydrolysis (DH) (Hoyle and Merritt 1994). Following hydrolysis, 20 mL of CWP and an equivalent volume of TCA (20%; w/v) were mixed until the final concentration of TCA was 10%. After the mixture was allowed to stand for 30 min, it was centrifuged at 8000 × g for 10 min. The DH was then calculated using the following formula after the supernatant's protein level was evaluated using the Kjeldahl method:

DH (%) = (soluble nitrogen in TCA 10% / total nitrogen in sample) × 100.

Antioxidant activity of CWP evaluation

Using 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity, the antioxidant capacity of CWP was evaluated at 0, 1, 2, 3, and 4 h. With minor modifications, the DPPH radical scavenging activity was ascertained (Göçer and Gülçin 2011; Ramadan et al. 2008). Each sample was diluted with 1 to 4 mL of 0.15 mM DPPH (in 95% ethanol) and vortexed vigorously. Before determining the absorbance at 517 nm, the reaction mixture was kept at room temperature for 30 min in the dark. The reduction in the absorbance of the DPPH radicals was used to compute the samples' radical scavenging capacity, which was determined using the formula:

Radical scavenging activity (%) = [(A control − A sample)/A control] × 100. A = absorbance at 517 nm.

Fish and rearing condition

Nile tilapia fish (16.02 ± 0.14 g) was gained from the Fish Research Unit at Zagazig University in Egypt and housed in 100-L glass aquaria. The fish were clinically examined for health status and then were adapted to the laboratory settings for 2 weeks during which

they were fed on a basal diet to apparent satiation three times daily. The aquaria were supplied with artificial aeration through air stones and pumps. Daily removal of the waste materials from the aquarium by siphoning with complete removal of the water 2 times/week was carried out. By the APHA (1998), the water parameters were assessed. The dissolved oxygen (DO) and water temperature were evaluated using an oxygen meter (970 portable DO meter, Jenway, London, UK). The pH was evaluated using a pH meter (Digital Mini-pH Meter, model 55, Fisher Scientific, Denver, CO, USA). The ammonia level was evaluated using the Ammonia MR checker (HANA Instruments Co., Egypt). The water parameters were as follows: (DO) (6.8 ± 0.4 mg/L), water temperature, pH (6.9 ± 0.4) (26.4 ± 1.5 °C), and ammonia (0.02 ± 0.001 mg/L).

Selection of the dietary level of CWP and carbonate alkalinity

A preliminary study was conducted using 225 Nile tilapia for the determination of the optimum dietary level of CWP for 30 days (Supplementary Material 1). The fish were divided into five groups in triplicates (45 fish/group; 15 fish/replicate) and were fed on five grading levels of CWP 0, 25, 50, 75, and 100 g kg^{-1} diet. The growth metrics (final body weight (FBW), total weight gain (TWG), specific growth rate (SGR), and average daily weight gain (ADWG)) were significantly improved by the CWP diets (25–100 g kg^{-1} diet) with the improvement of the feed conversion ratio (FCR) relative to the control. According to the broken-line regression model, CWP75 was the best dietary level based on the data of TWG and FCR, so a dietary level of 75 g kg^{-1} diet CWP was used for our experimental study. In addition, we used the carbonate alkalinity level of 23.8 mmol/L according to the previous study (Cheng et al. 2022) in Nile tilapia.

Diet preparation and experimental setup

Two diets were developed to meet the dietary needs of Nile tilapia (NRC 2011). The control diet was a basal diet (Table 1) with no dietary supplements, while the CWP diet was a basal diet that contained 75 g/kg of CWP. Using a meat mincer, the diet components were machinely combined and then formed into 1.5-mm pellets. After that, they were air-dried at 25 °C for 24 h with frequent rotation. Finally, the pellets were packed in a refrigerator at 4 °C until they were needed. A total of 160 fish were split into four groups, with four replicates for each group (10 fish per replicate; 40 fish total). The first control group (C) and the second (CWP) were raised in freshwater (pH of 7.19 and carbonate alkalinity of 1.4 mmol/L) and fed basal diets enriched with 0 and 75 g CWP/kg diet, respectively. The third (ALK) and the fourth (CWP + ALK) groups were given the basal diet and the CWP-supplemented diet, respectively, and they were kept in alkaline water (pH = 8.65; carbonate alkalinity = 23.8 mmol/L). For 30 days, the fish were kept in experimental settings and fed prepared diets. The diets were introduced 3 times/day (10 a.m., 1 p.m., and 4 p.m.) until apparent satiation during the trial period. Every day, fish deaths and clinical signs were noted.

Table 1 Formulation and proximal chemical composition of the basal diet (g/kg on a dry basis)

	Basal diet
Ingredients	
Fish meal	180
CWP	0
Fish oil	60
Ground yellow corn	243
Soybean meal 44%	255
Corn gluten 60% CP	110
Wheat	50
Wheat bran	90
Premix[#]	12
Calculated chemical analysis	
Crude protein, CP	336.2
Fat	94.6
NFE[*]	385.6
Crude fiber	37.4
Calcium	10.4
Lysine	18.3
Methionine	7.1
Available phosphorus	9.1
DE (Kcal/kg)[**]	2907.3
CP/DE (mg/Kcal)	115.6

[*]*NFE* "Nitrogen free extract" = 1000 − (g/kg crude protein + fat + ash + crude fiber)

[**]Digestible energy (DE) was calculated by applying the coefficient of 0.75 to convert gross energy to digestible energy

[#]Premix: Each 1 kg of premix contains vitamin A 550,000 IU, vitamin D 110,000 IU, vitamin E 11,000 mg, vitamin K 484 mg, vitamin C 50 g, vitamin B1 440 mg, vitamin B2 660 mg, vitamin B3 13,200 mg, vitamin B5 1100 mg, vitamin B6 1045 mg, vitamin B9 55 mg, choline 110,000 mg, biotin 6.6 mg, iron 6.6 g, copper 330 mg, manganese 1320 mg, zinc 6.6 g, selenium 44 mg, and iodine 110 mg

Sampling

Fish (12/group) were fasted for 24 h at the termination of the experiment before blood sampling. The average size of the fish at the end of the experiment was 31.69 ± 2.83 g. The fish were sedated using a 100 mg/L benzocaine solution following the Neiffer and Stamper (2009) method. The caudal blood vessels were pierced to collect blood samples. To extract the serum, the samples were centrifuged for 10 min at 1075 × g. The serum was then kept at − 20 °C to evaluate the biochemical parameters. The fish were killed using benzocaine solution (400 mg/L) (Tran-Duy et al. 2008) to obtain the tissue samples. Fifty milligrams of liver tissue (12/group) was sampled and preserved in 1 mL of QIAzol (Qiagen, Germany). After that, the tissue was stored at − 80 °C in an RNAlater (Sigma-Aldrich, Poole, UK) until an assay for gene expression was conducted. Furthermore, liver tissue (12 samples/group) was taken and kept for histological analysis in 10% neutral buffered formalin (El Gomhouria Co. Egypt).

Biochemical indices

The technique of Fossati and Prencipe (1982) was applied to measure the serum triglycerides (TG) using the TG quantification kit. TGs were broken down into glycerol and free fatty acids. Following its release, the glycerol underwent oxidation to yield a product that reacted with a probe to produce a color that was detected at 570 nm by a spectrophotometer (APEL, PD-303 UV, Kawaguchi, Japan). As per Allain et al. (1974), the measurement of cholesterol (CHO) was conducted using the cholesteryl ester enzyme assays. The techniques outlined by Henry (1964) and Doumas et al. (1971) were applied to determine serum total protein (TP) and albumin (ALB), respectively. ALB's value was subtracted from the TP value to determine serum globulin (GLO). Using commercial kits (Biodiagnostic Co., Giza, Egypt), serum alanine (ALT) and aspartate aminotransferase (AST) levels were assessed in compliance with Reitman and Frankel (1957) recommendations. By mixing 0.25 mL of buffered L-alanine with 50 mL of supernatant, the amount of ALT was determined. The mixture was then incubated for 30 min at 37 °C. This mixture was mixed with 0.25 mL of 2,4-dinitrophenylhydrazone (2,4-DNPH) and allowed to stand at room temperature for 20 min. Next, 2.5 mL of sodium hydroxide (0.4 (N) NaOH) was added, well combined, and allowed to stand for 10 min. At 505 nm, the OD was measured in a UV spectrophotometer. To determine the AST level, a 50-mL sample was mixed with 0.25 mL of buffered aspartate, and the mixture was then incubated for 60 min at 37 °C. Following the addition of 0.25 mL of 2,4-DNPH, the mixture was allowed to stand for 20 min at room temperature. Next, 2.5 mL of 0.4 (N) NaOH was added, well combined, and allowed to stand for 10 min. At 505 nm, the OD was measured using a UV spectrophotometer (Tongfang, Inc., China). According to Molina et al. (2005), acid phosphatase (ACP) was used as a marker enzyme to measure the ALP at 400 nm OD. Gamma-glutamyltransferase (GGT) activity was determined using the kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's instructions for spectrophotometry at 450 nm. Total bilirubin (T-BLR) was assessed according to Ngashangva et al. (2019) which relies on the reaction of bilirubin with diazotized sulfanilic acid (DSA) producing a red color. The absorbance value was measured at 546 nm wavelength. Serum high-density lipoprotein (HDL) and low-density lipoprotein (LDL) were assessed with the kits using an automatic biochemistry analyzer (CHEMIX-800, Sysmex Corporation, Kobe, Japan) following the manufacturer's instructions.

Hepatic oxidant/antioxidant indices

The oxidant/antioxidant parameters were estimated spectrophotometrically in the hepatic homogenate. Utilizing the kits (Bio-Diagnostic, Cairo, Egypt), the superoxide dismutase (SOD) (catalog no. SD2521), catalase (CAT) (catalog no. CA2517), reduced glutathione (GSH) (catalog no. TA2511), and malondialdehyde (MDA) (catalog No. MD2529) were assessed. SOD was assessed based on the ability of the enzyme to prevent the phenazine methosulphate–mediated reduction of nitroblue tetrazolium dye at 560 nm wavelength. The enzymatic reaction mixture, which contained samples, potassium phosphate (pH 7.0), and hydrogen peroxide (H_2O_2), was used to calculate CAT. The molar attenuation coefficient of H_2O_2 was determined using a UV–VIS spectrophotometer set at 240 nm. The basis for determining GSH was the reduction of 5,5′dithiobis (2-nitrobenzoic acid, DTNB), dissolved in 25 mM PBS, pH 7.0, by the GSH to produce a yellow product measured at 405

nm. The MDA level was assessed using thiobarbituric acid method. At 535 nm, the reactive components of thiobarbituric acid were measured and then expressed in terms of MDA generated.

Real-time quantitative polymerase chain reaction (RT-qPCR) analysis

RNA was obtained from frozen liver specimens utilizing the QIAzol (Qiagen, Germany). Using the Quantitect® Reverse Transcription kit (Qiagen, Germany), the extracted RNA was utilized to obtain the first strand of cDNA. The autophagy-related gene 5 (*ATG-5*), autophagy-related gene 7 (*ATG-7*), autophagy-related gene 13 (*ATG-13*), mitogen-activated protein kinase (*P38*), c-Jun NH terminal kinase (*JNK*), mitogen-activated protein kinase (*MAPK-1*), mechanistic target of rapamycin (*mTOR*), and cathepsin B with ubiquitin-conjugating enzyme (*ubce2*) as a housekeeping gene were assessed (Table 2). Using a QuantiTect® SYBR® Green PCR kit (Qiagen, Germany), the qPCR analysis was carried out in a Rotor-Gene Q instrument with thermocycler conditions: 95 °C for 10 min, followed by 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The relative mRNA expression pattern of every gene was calculated following the comparative $2^{-\Delta\Delta Ct}$ protocol (Schmittgen and Livak 2008).

Table 2 Primer list

Gene	Sequence	TM	Primer efficiency	Size	Accession no
ubce2	CTCTCAAATCAATGCCACTTCC	57.63	102.5	130	XM_003460024.5
	CCCTGGTGGAGGTTCCTTGT	61.43			
ATG-5	TTATGTGCCACTGGGAGCAG	60.04	98.51	152	XM_003450274.5
	CTCACCCTGGGTAACAGCAG	60.04			
ATG-7	ACCCTGTTTCCGTACGGTTC	59.97	99.5	156	XM_025907253.1
	ACGTACCGTCAGTTTTCGCT	59.97			
ATG-13	TACAGGGCTCCCGATGAAGA	60.03	98.42	112	XM_003455882.5
	TGGAGGCGTACAGCTGAGA	60.3			
P38	GTTTCCCGGCACTGACCATA	60.04	100.5	98	XM_003448218.5
	CCTCATAGCTGGGCATCCTG	59.96			
JNK	AAAGCGTGGTGGAGTCTCTG	59.97	102.52	104	XM_005455390.4
	CTCCCTCTCAGCCTCTTCCT	60.03			
MAPK-1	CTTTGGTTTGGCTCGTGTGG	59.97	98.45	171	XM_003444474.5
	TTGGACAGCATCTCAGCCAG	60.04			
mTOR	CGAATAGCAATTAGGCCGTCCA	60.81	94.68	75	XM_003449131.5
	GTCCACTGACAAACTGCTGC	59.69			
Cathepsin B	AAATGGCACTCCCTACTGGC	60.03	94.85	92	MF422074.1
	AGTGATCTGAACCACGCAGG	60.04			

TM melting temperature, *ubce2* ubiquitin-conjugating enzyme, *ATG-5* autophagy-related gene 5, *ATG-7* autophagy-related gene 7, *ATG-13* autophagy-related gene 13, *P38* mitogen-activated protein kinase, *JNK* c-Jun NH terminal kinase, *MAPK-1* mitogen-activated protein kinase, *mTOR* mechanistic target of rapamycin

Histopathological analysis

Liver samples (12/group) were taken following the procedures detailed by Meyers (2009). Promptly, these specimens were subjected to formalin fixation and paraffin embedding, sectioning at 5 µm thick, and hematoxylin and eosin staining as stated by Suvarna et al. (2018). Next, a comprehensive histological assessment of the liver tissue sections was carried out to calculate the frequencies of the histological alterations (alt), if any, and precisely determine the liver index for each group following the protocol designed by Bernet et al. (1999). The alt were recorded in 10 high-power fields (40×) chosen randomly for each fish (100 fields for each group). The lesion frequencies were calculated using the formula:frequency $(F) = \frac{N(\text{alt})}{N(\text{total})} \times 100$, where N (alt) is the number of times of appearance of the alteration and N (total) is the total number of 40× microscopic fields in the group (100). The liver index for each group (the higher the liver index, the worse the pathological condition) was calculated from the formula: Liver index $= \Sigma_{\text{rp}}\ \Sigma_{\text{alt}}\ (a_{\text{rp alt}} \times w_{\text{rp alt}})$. A light microscope was used to examine the stained slides, and any changes to the histology were noted. The AmScope ToupView v4.8.15934 software (AmScope, Irvine, CA, USA) was used for all of the microscopic morphometric measurements.

Statistical analysis

To make sure that the results obtained were regularly distributed, the Shapiro–Wilk test was applied. The experimental results were statistically examined using a one-way analysis of variance (ANOVA) utilizing IBM's SPSS version 17. When comparing the means of various groups, Duncan's post hoc test was used, and the statistical significance was acceptably $P < 0.05$. The results of the analysis were shown as means ± standard error (SE).

Results

Characterization of CWP

Trypsin hydrolyzed CWP at a 1:200 E/S ratio (E/S) under ideal conditions for 4 h. We estimated the DH and the antioxidant activity in CWP at varying intervals (1–4 h) and presented the results in Fig. 1. The DH gradually increased from 13 after 1 h to 32% after 4 h (Fig. 1A). There was a parallel increase in antioxidant capacity from 48% at 1 h to 80% at 4 h (Fig. 1B). The tryptic hydrolysates of CWP-4h (highest antioxidant activity) were chosen for the feeding experiment.

Lipid profile results

As shown in Table 3, TG and CHO levels were substantially higher ($P < 0.05$) in the ALK group than the CWP + ALK group relative to the C group (control), with no substantial variation between the C and CWP groups. A substantial rise ($P < 0.05$) in the

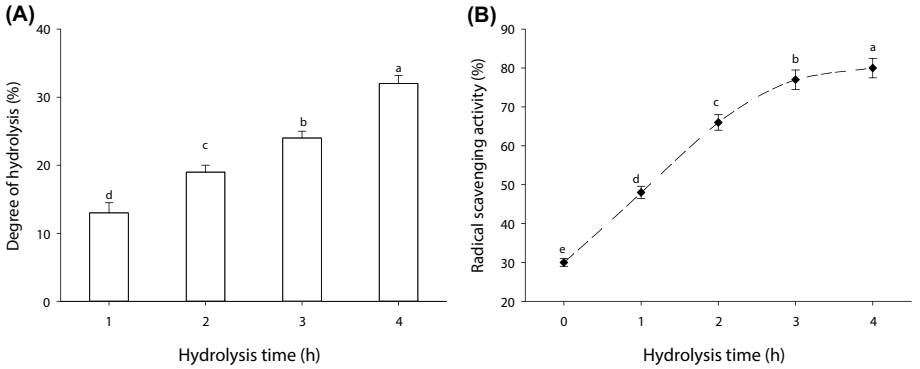

Fig. 1 Degree of hydrolysis (DH %) of CWPs (E/S = 1:200) during 4 h at 37 °C and pH 8 (**A**), and DPPH free radical scavenging activity of CWPs hydrolysates) 500 µg/mL) produced by trypsin (E/S = 1:200) at 37 °C and pH 8 at different time intervals (**B**). Different small letters indicate significant differences following Duncan's post hoc test ($P < 0.05$)

HDL with a substantial decrease ($P < 0.05$) in the LDL was noted in the CWP group relative to the C group. On the contrary, the ALK group showed a substantial decrease in the level of HDL and a substantial rise in the LDL followed by the CWP + ALK group relative to the C group.

Table 3 Effect of dietary supplementation of camel whey protein hydrolysates (CWP) on the biochemical parameters of Nile tilapia exposed to alkaline stress for 30 days

Parameters	C	CWP	ALK	CWP + ALK	*P*-value
TG (mg/dL)	114.11 ± 4.04^{a}	113.99 ± 2.24^{a}	146.77 ± 4.38^{b}	136.637 ± 3.79^{c}	< 0.001
CHO (mg/dL)	130.84 ± 5.00^{a}	132.19 ± 1.90^{a}	176.84 ± 5.27^{b}	150.85 ± 5.57^{c}	0.04
HDL (mg/dL)	54.21 ± 0.71^{a}	63.36 ± 0.64^{b}	42.65 ± 0.84^{c}	48.20 ± 1.07^{d}	0.01
LDL (mg/dL)	76.63 ± 5.21^{a}	68.83 ± 5.48^{b}	134.19 ± 0.81^{c}	$102.65 \pm 0.5.08^{d}$	0.04
TP (g/ dL)	3.33 ± 0.07^{a}	3.76 ± 0.04^{b}	2.12 ± 0.06^{c}	2.86 ± 0.05^{d}	< 0.001
ALB (g/ dL)	1.72 ± 0.02^{a}	1.81 ± 0.06^{b}	1.33 ± 0.02^{c}	1.78 ± 0.05^{d}	< 0.001
GLB (g/ dL)	1.61 ± 0.04^{a}	1.95 ± 0.02^{b}	0.79 ± 0.11^{c}	1.08 ± 0.02^{d}	< 0.001
ALT (U/L)	9.61 ± 0.36^{a}	9.81 ± 0.61^{a}	15.11 ± 0.63^{b}	12.74 ± 0.53^{c}	< 0.001
AST (U/L)	37.87 ± 4.54^{a}	35.37 ± 3.31^{a}	84.43 ± 1.87^{b}	62.74 ± 3.27^{c}	< 0.001
ALP (U/L)	130.55 ± 3.32^{a}	133.64 ± 4.84^{a}	181.32 ± 1.85^{b}	165.43 ± 4.36^{c}	< 0.001
GGT (U/L)	2.90 ± 0.08^{a}	2.89 ± 0.13^{a}	4.94 ± 0.32^{b}	3.41 ± 0.36^{c}	0.001
T-BLR (mg/dL)	0.50 ± 0.01^{a}	0.53 ± 0.02^{a}	0.65 ± 0.01^{b}	0.58 ± 0.01^{c}	0.001

Variation in the data was expressed as means ± *SE*

TG triglycerides, *CHO* cholesterol, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *TP* total protein, *ALB* albumin, *GLB* globulin, *ALT* serum alanine aminotransferase, *AST* serum aspartate aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma-glutamyltransferase, *T-BLR* total bilirubin, *C* control group was fed basal diet and reared in freshwater, *CWP* group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater, *ALK* and *CWP + ALK* fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

[a,b,c]Mean values in the same row with different superscripts differ significantly $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group)

Protein profile results

A substantial rise ($P < 0.05$) in the TP, ALB, and GLB was noted in the CWP group relative to the C group. On the contrary, these variables considerably declined in the ALK group comparable to the C group, which were modulated in the CWP + ALK group (Table 3).

Liver function indices results

No significant alteration was noticed in the ALT, AST, ALP, GGT, and T-BLR between the C and CWP groups (Table 3). These indices were significantly ($P < 0.05$) increased in the ALK group relative to the C group, which were modulated in the CWP + ALK group.

Oxidant-antioxidant results

The SOD, CAT, and GSH activities were considerably increased ($P < 0.05$) in the CWP group comparable to the C group. The activity of these enzymes was considerably lowered in the ALK group comparable to the C group, which was modulated in the CWP + ALK group. There was no considerable variation in the MDA level between the C and CWP groups, while the level of MDA ($P < 0.05$) was considerably higher in the ALK group relative to the C group, which was modulated in the CWP + ALK group (Table 4).

Gene expression results

The *ATG-5* and *ATG-13* expression was considerably ($P < 0.05$) down-regulated in the ALK group relative to the C group, with no considerable variation between the C and CWP groups. Moreover, the *ATG-7* expression was up-regulated ($P < 0.05$) in the CWP group

Table 4 Effect of dietary supplementation of camel whey protein hydrolysates (CWP) on the oxidant/antioxidant functions of Nile tilapia exposed to alkaline stress for 30 days

Parameters	C	CWP	ALK	CWP + ALK	*P*-value
SOD (U g^{-1})	146.34 ± 5.32^{a}	199.18 ± 5.24^{b}	69.19 ± 1.78^{c}	109.86 ± 5.22^{d}	< 0.001
CAT (U g^{-1})	7.17 ± 0.60^{a}	10.18 ± 0.54^{b}	1.88 ± 0.14^{c}	3.91 ± 0.18^{d}	< 0.001
GSH (U g^{-1})	152.40 ± 3.76^{a}	184.43 ± 6.34^{b}	68.97 ± 2.75^{c}	103.65 ± 6.49^{d}	< 0.001
MDA (nmol mg^{-1})	0.68 ± 0.05^{a}	0.79 ± 0.07^{a}	3.03 ± 0.09^{b}	2.99 ± 0.09^{c}	< 0.001

Variation in the data was expressed as means ± *SE*

SOD superoxide dismutase, *CAT* catalase, *GSH* reduced glutathione, *MDA* malondialdehyde, *C* control group was fed basal diet and reared in freshwater, *CWP* group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater, *ALK* and *CWP + ALK* fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

[a,b,c]Mean values in the same row with different superscripts differ significantly $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group)

and down-regulated in the ALK group comparable to the C group. The *ATG-5*, *ATG-7*, and *ATG-13* expressions were considerably modulated in the CWP + ALK group relative to the ALK group (Fig. 2). Figure 3 shows a considerable rise ($P < 0.05$) in the expression of *P38*, *JNK*, and *MAPK-1* in the ALK group followed by the CWP + ALK group relative to the C group, with no considerable variation between the C and CWP group in the *P38* expression. The expression of *mTOR* (Fig. 4) was substantially up-regulated ($P < 0.05$) in the ALK group, and then the CWP + ALK group relative to the C group, with no considerable variation between the C and CWP group. The expression of cathepsin B was increased ($P < 0.05$) in the CWP group relative to the C group (Fig. 4), and cathepsin B expression was decreased in the ALK group comparable to the C group, which modulated in the CWP + ALK group.

Histopathological results

The C and CWP groups showed normal histology of the tilapia's hepatopancreas without any histological alterations (Fig. 5 (A) and (B)). The ALK group exhibited numerous alterations, primarily of a degenerative and inflammatory nature. The most encountered lesions included acute cellular swelling, single-cell necrosis, focal lytic necrosis, tiny multifocal lipidosis (Fig. 5 (C1)), vascular congestion with inflammatory cell infiltrates particularly with the wandering granular eosinophilic cells (Fig. 5 (C2)), and hyperplasia of the melanomacrophage centers (Fig. 5 (C3)). CWP showed moderate hepatoprotective effects against the hepatopathy induced by the alkaline water stress. Although similar lesions were present in the tissue sections of the CWP + ALK group (Fig. 5 (D1), (D2), and (D3)) compared to the ALK group, they were less frequent and less severe. The statistical analysis for the hepatopathic alterations in all groups and the liver indices was presented in Table 5.

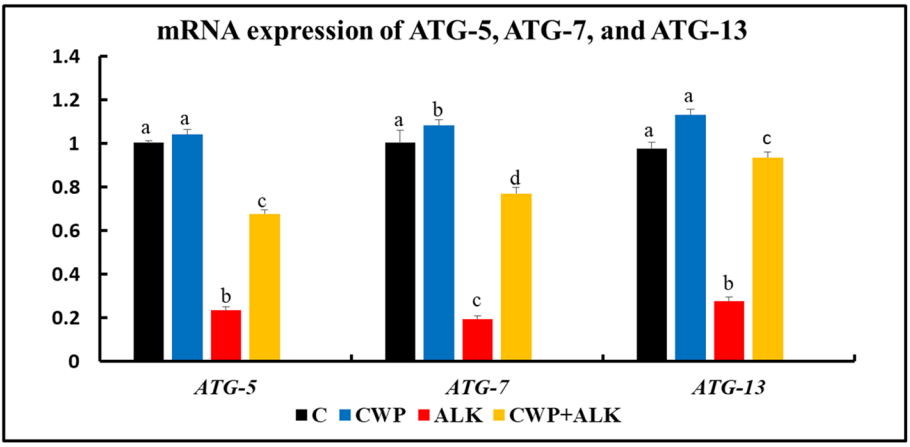

Fig. 2 mRNA expression of autophagy-related genes (*ATG-5*, *ATG-7*, and *ATG-13*) of Nile tilapia exposed to alkaline stress for 30 days and fed on camel whey protein (CWP) diet. Values are represented as the mean ± SE. The means within the same bar carrying different superscripts are significant at $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group). C, control group was fed basal diet and reared in freshwater; CWP, group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater; ALK and CWP + ALK were fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

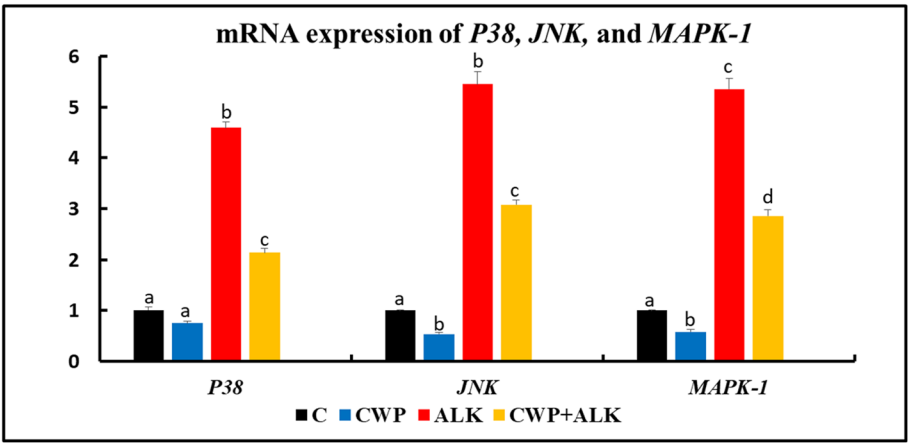

Fig. 3 mRNA expression of mitogen-activated protein kinase (*P38*), c-Jun NH terminal kinase (*JNK*), and mitogen-activated protein kinase (*MAPK-1*) of Nile tilapia exposed to alkaline stress for 30 days and fed on camel whey protein (CWP) diet. Values are represented as the mean ± SE. The means within the same bar carrying different superscripts are significant at $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group). C, control group was fed basal diet and reared in freshwater; CWP, group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater; ALK and CWP + ALK were fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

Discussion

A wide area of the world's water resources is made up of alkaline water, which has an impact on the physiological functions of aquatic species, including tissue water content,

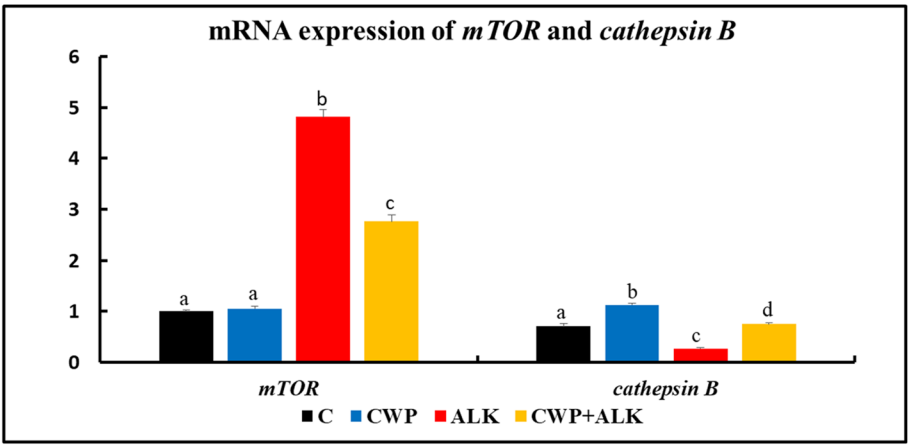

Fig. 4 mRNA expression of mechanistic target of rapamycin (*mTOR*) and cathepsin B of Nile tilapia exposed to alkaline stress for 30 days and fed on camel whey protein (CWP) diet. Values are represented as the mean ± SE. The means within the same bar carrying different superscripts are significant at $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group). C, control group was fed basal diet and reared in freshwater; CWP, group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater; ALK and CWP + ALK were fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

hemolymph osmolality pressure, and survival (Su et al. 2020). One of the main stresses on fish is alkaline water (Shang et al. 2021). In this research, we looked at the impact of carbonate alkalinity on the liver tissue as a main organ of metabolism and detoxification, as well as the potential role of CWP in mitigating this alkaline stress. Our results showed that alkaline stress elevated the serum TG, CHO, and LDL levels in Nile tilapia. Alkaline stress was reported to activate the super pathway of cholesterol biosynthesis in Amur ide (*Leuciscus waleckii)* (Xu et al. 2013) and serum triglycerides level in Amur minnow (*Phoxinus lagowskii*) (Zhou et al. 2024). Elevated cholesterol and triglycerides in fish are regarded as indicators of poor health (Kikuchi et al. 2009; Rahimnejad et al. 2021). Increased TG, CHO, and LDL levels are attributed to the mobilization of lipids via oxidation or a process of lipid molecules being gradually reconstituted from the synthesis site for use later on. These lipid molecules are typically employed to combat stress. An elevated lipid profile can result from a disruption in lipid metabolism or from poor blood clearance, which can support liver dysfunction. Membrane alterations or damage lead to a subsequent change in the lipid profile (Javed and Usmani 2015). Noteworthy, the CWP diet improved the lipid profile of Nile tilapia reared under alkaline stress by lowering the TG, CHO, and LDL levels and increasing the HDL level. These outcomes could related to the hypolipidemic effect of CWP active peptides (Kilari et al. 2021). In addition, CWP peptides may influence lipid metabolism by inhibiting pancreatic lipase and CHO esterase enzyme activity (Baba et al. 2021).

Fish nutritional and physiological status is frequently correlated with blood protein levels (Maita 2007). During times of malnutrition or stress, blood protein levels diminish as a result of proteolysis or oxidation of amino acids (Peres et al. 2014). In this study, alkaline stress reduced the blood protein profile (TP, ALB, and GLB). This reduction could be brought on by impaired synthesis and nonspecific proteolysis of serum proteins to meet increased energy demands during stress, as well as vascular leakage of serum proteins (Kumar et al. 2018; Singh et al. 2019). The protein profile of the fish exposed to alkaline conditions was enhanced in this study by the CWP diet. These outcomes could be explained by the CWP richness of amino acids like tryptophan, phenylalanine, tyrosine, histidine, and cysteine (Osman et al. 2021a; Salami et al. 2010; Elias et al. 2005).

ALT and AST are indicators of a shift in physiological state or stress which function on the link between proteins and carbohydrates in metabolism. These enzymes have been utilized to demonstrate damage to fish and liver tissues (Asztalos and Nemcsok 1985). ALP is involved in the production and release of specific enzymes, cell differentiation, growth, metabolism of carbohydrates, and protein synthesis. It has been observed that fish that are stressed or injured have higher levels of this enzyme (Al-Khshali and Al Hilali 2019). GGT is a liver enzyme that has an extensive distribution in cells that are involved in bile secretion and absorption. It is produced by the microsomes. It serves as a useful indicator in the laboratory for any damage to the liver cells (Ovie et al. 2012). Heme, a substance derived from red blood cell hemoglobin or other hemoproteins, is broken down to produce bilirubin. Bilirubin is eliminated through urine and feces after being secreted through the bile. Damage to liver function results in elevated blood bilirubin (Hastuti et al. 2019). In this study, the alkaline-exposed fish showed an elevation of ALT, AST, ALP, GGT, and T-BLR. The results of elevated liver enzymes were confirmed by the histopathological investigation of the hepatic tissue of the alkaline-exposed fish in this study. The liver of alkaline-exposed fish revealed numerous alterations, primarily of a degenerative and inflammatory nature. In addition, An increase in metabolic transport may be the cause of elevated hepatic enzyme levels, which could ultimately cause a change in the alkaline-exposed fish's energy metabolism and biosynthesis pathway (Oyeniran et al. 2021). Elevated liver function enzymes (ALT,

Table 5 Effect of dietary supplementation of camel whey protein hydrolysates (CWP) on the hepatic histology of Nile tilapia exposed to alkaline stress for 30 days

Lesions			C		ALK		CWP		CWP + ALK	
Reaction pattern	Histological alteration	*W*	*F*	Index	*F*	Index	*F*	Index	*F*	Index
Inflammatory alterations	Leukocytic infiltration	2	0	0	45	1 ± 0.44	0	0	23	0.40 ± 0.26
	Granuloma formation	2	0	0	1	0.40 ± 0.00	0	0	0	0.00 ± 0.00
Circulatory alterations	Congestion	1	0	0	62	1.10 ± 0.37	0	0	29	0.60 ± 0.33
	Hemorrhages	2	0	0	4	0.10 ± 0.01	0	0	0	0.10 ± 0.01
	Edema	1	0	0	4	0.20 ± 0.02	0	0	0	0.00 ± 0.00
	Thrombi formation	3	0	0	0	0.00 ± 0.00	0	0	0	0.00 ± 0.00
Regressive alterations	Cellular swelling	1	0	0	43	0.90 ± 0.34	0	0	30	0.60 ± 0.33
	Vacuolation foci	2	0	0	7	0.40 ± 0.02	0	0	3	0.20 ± 0.03
	Single-cell necrosis	2	0	0	32	0.60 ± 0.30	0	0	19	0.40 ± 0.26
	Focal lytic necrosis	3	0	0	11	0.30 ± 0.02	0	0	2	0.30 ± 0.03
	Cholestasis	2	0	0	2	0.20 ± 0.02	0	0	1	0.00 ± 0.00
Progressive alterations	Fibrosis	2	0	0	0	0.00 ± 0.00	0	0	0	0.00 ± 0.00
	Hepatocyte hyperplasia	2	0	0	0	0.00 ± 0.00	0	0	0	0.00 ± 0.00
	Cholangiocyte hyperplasia	2	0	0	0	0.20 ± 0.01	0	0	0	0.00 ± 0.00
	Melanomacrophage centers hyperplasia	2	0	0	41	1.4 ± 0.66	0	0	17	0.40 ± 0.26
Neoplastic alterations	Adenomas, carcinomas, and/or sarcomas	3	0	0	0	0	0	0	0	0
Liver index			0		0.42		0		0.19	

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $P < 0.05$ (one-way ANOVA; Duncan's post hoc test) ($n = 12$/group)

F lesion frequency, *W* importance factor, *C* control group was fed basal diet and reared in freshwater, *CWP* group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater, *ALK* and *CWP + ALK* fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

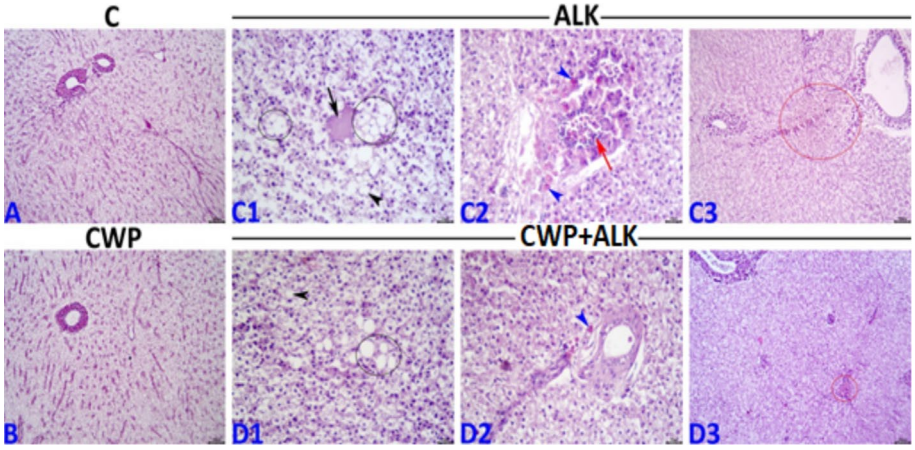

Fig. 5 Representative H&E-stained light photomicrographs of the hepatic tissue sections showing normal hepatopancreas in the C (A) and CWP (B) groups. The hepatic tissue of the ALK group shows single-cell necrosis (black arrowhead), focal lytic necrosis (black arrow), tiny multifocal lipidosis (black ellipses) (C1), vascular congestion (red arrow) with inflammatory cell infiltrates particularly with the wandering granular eosinophilic cells (blue arrowheads) (C2), and hyperplasia of the melanomacrophage centers (red ellipse) (C3). Noticeable reductions in the severity of the lesions were seen in the hepatic tissue sections of the CWP + ALK group which shows mild lipidosis (black ellipse) (D1), few wandering granular eosinophilic cells (blue arrowhead) (D2), and mild hyperplasia of the melanomacrophage centers (red ellipse) (D3). C, control group was fed basal diet and reared in freshwater; CWP, group was fed basal diet supplemented with 75 g/kg diet CWP and reared in freshwater; ALK and CWP + ALK were fish groups fed basal diets supplemented with 0 and 75 g/kg diet CWP, respectively, and reared in alkaline water

AST, and ALP) were previously reported in Chinese mitten crab (*Eriocheir sinensis)* as a result of alkaline stress (Li et al. 2022). Noteworthy, the CWP diet modulated the liver function enzymes of alkaline-exposed fish and improved the hepatic histological picture. These outcomes could be attributed to the hepatoprotective effect of CWP due to its amino acid content with powerful antioxidant activity (Osman et al. 2021a). In addition, Kilari et al. (2021) reported that CWP has a hepatoprotective effect as a result of its active peptides which ameliorate liver damage in rats.

Reactive oxygen species (ROS) are produced in excess when antioxidant enzyme activity is low, leading to oxidative stress (Chowdhury and Saikia 2020). Lipid peroxidation results in the generation of MDA (Özok 2020). In this research, alkaline stress exhibited higher MDA levels and lower antioxidant enzymes (SOD, CAT, and GSH). Similar oxidative stress was noted in the hybrid tilapia (*O. niloticus* × *O. aureus*) under alkaline stress (Han et al. 2016). CWP diet significantly improved the antioxidant enzyme activity and lowered the MDA level in the liver of alkaline-exposed fish. The antioxidant properties of CWP were confirmed in this study through high DPPH radical scavenging activity (80%). Similar results were obtained by Alsaloom (2024) who reported that CWP has antioxidant capacity through high DPPH radical scavenging activity (70%). The antioxidant properties of CWP could be related to the availability of the bioactive peptides; these peptide fractions have scavenging behavior through their ability to act as electrons or hydrogen donors in free radical reactions (Alsaloom 2024; Al-Shamsi et al. 2018). Furthermore, Abd and Rahim (2020) stated that CWP has some electron-donating molecules which interact with free radicals and prevent the

radical chain reaction. In addition, CWP has cysteine, tyrosine, histidine, phenylalanine, and tryptophan in their structure, which can scavenge oxygen-free radicals (Osman et al. 2021a; Salami et al. 2010; Elias et al. 2005).

In this study, we analyzed various gene expressions associated with key pathways such as autophagy and inflammation. We aimed to elucidate the mechanisms by which alkaline stress may influence the hepatic microenvironment. In eukaryotes, autophagy functions as an intracellular degradation system, a non-lethal response to preserve cellular homeostasis, and a stress adaptation mechanism that averts cell death in the event of shifting internal and external circumstances (Tang et al. 2018). Autophagy also contributes to the clearance of intracellular proteins and organs, anti-aging processes, cell death, and tumor suppression. Numerous illnesses can be brought on by abnormal levels of autophagy (Tanida 2011). Autophagy pathways are controlled by intracellular signal transduction pathways, such as the *mTOR* signaling pathway (Xia et al. 2019). Among the autophagy genes are *ATG-5*, *ATG-7*, and *ATG-13*. *MAPK* superfamily is a member of serine/threonine protein kinase and has crucial roles in the way that cells react to external stimuli (Qu et al. 2020). The key players in signal transduction pathways, *P38* and *JNK*, are primarily activated by inflammatory cytokines and environmental stress (Xu et al. 2020). *P38* can regulate stress responses and is essential for shielding organisms from environmental dangers (Zhang et al. 2019). *JNKs* are essential for controlling inflammatory reactions, cell death, and stress response (Roy et al. 2008). *mTOR* is a serine-threonine protein kinase which considered an important component in determining cellular nutrition and energy status (Jiang et al. 2013). *mTOR* signaling pathway is essential for controlling the production of autophagy and apoptosis (Liang et al. 2020). One of the common members of the cysteine protease family is cathepsin B. It possesses both endopeptidase and exopeptidase activity. It is crucial for the presentation of antigens, their degradation, apoptosis, the inflammatory response, and the physiological processes involved in many diseases (Shen et al. 2021). In this study, alkaline stress up-regulated *mTOR* and *MAPK*-related genes (*P38*, *JNK*, and *MAPK-1*) expression and down-regulated the autophagy-related genes (*ATG-5*, *ATG-7*, and *ATG-13*) and cathepsin B expression. Alkaline stress–induced up-regulation of *mTOR* expression may help preserve energy and increase survival during protracted stressful situations (Teets et al. 2012). Activation of the *mTOR* expression was previously reported as a result of alkaline stress in Amur ide (*Leuciscus waleckii*) (Xu et al. 2013). In addition, activation of the *MAPK* pathway was previously reported as a result of alkaline stress in the Chinese mitten crab (*Eriocheir sinensis)* (Wang et al. 2023). Numerous cellular processes linked to pathological conditions, including apoptosis, the inflammatory response, and immunological disorders, are mediated by the expression of cathepsin B (Shen et al. 2021). Noteworthy, the CWP diet in this study modulated the detrimental effect of alkaline stress through modulation of the autophagy, *MAPK*, *mTOR*, and cathepsin B expression in the alkaline-exposed fish. These results confirmed the anti-stress properties of CWP. Overall, CWP can be applied as a feed additive in the Nile tilapia diet for mitigating the alkaline stress, which gives a novel proscriptive for rearing this species in alkaline water.

Conclusion

Alkaline stress disrupted the metabolism in the Nile tilapia liver through elevation of the lipid profile and function enzymes by lowering the protein profile. In addition, there is the activation of the mTOR and MAPK pathway with down-regulation of the autophagy and cathepsin B expression in the hepatic tissue. Dietary supplementation with 75 g/kg

diet CWP modulated the liver function enzymes and lipid profile with improvement of the protein profile of the alkaline exposed fish, as well as the modulation of the hepatic transcriptomic profile of Nile tilapia during alkalinity exposure. We proposed a new dietary addition (75 g CWP/kg diet) for ameliorating the stress consequences in Nile tilapia reared under alkaline conditions.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10499-024-01642-w.

Author contribution REI, GEE, MYM, AAA, EMY, YMA, TK, AO, MMMM, SJD, and AAM: conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, and visualization. REI: writing original draft. REI, YMA, and AAM: writing review and editing. All authors read, reviewed, and approved the final manuscript.

Funding This work was supported by the Researches Supporting Project (RSPD2024R700), King Saud University, Riyadh, Saudi Arabia.

Data availability All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval The Institutional Animal Care and Use Committee of Zagazig University, Egypt, approved the experimental protocol (ZU-IACUC/2/F/393/2023), and all applicable institutional standards were followed when caring for and using animals in this study.

Consent to participate All authors have participated in this work.

Consent for publication All authors reviewed and approved the manuscript for publication.

Conflict of interest The authors declare no competing interests.

References

Abd EL, Rahim AM (2020) Antioxidant and antimicrobial activities of enzymatic hydrolysates of camel's milk whey protein and casein. J Food Dairy Sci 11(2020):45–50

Abdel-Hamid M, Goda HA, De Gobba C, Jenssen H, Osman A (2016) Antibacterial activity of papain hydrolysed camel whey and its fractions. Int Dairy J 61:91–98

Abdel-Hamid M, Otte J, De Gobba C, Osman A, Hamad E (2017) Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. Int Dairy J 66:91–98

Abdel-Hamid M, Romeih E, Saporito P, Osman A, Mateiu RV, Mojsoska B, Jenssen H (2020) Camel milk whey hydrolysate inhibits growth and biofilm formation of *Pseudomonas aeruginosa* PAO1 and methicillin-resistant *Staphylococcus aureus*. Food Control 111:107056

Al-Khshali MS, Al Hilali HA (2019) Some physiological changes (ALP, AST and ALT) of common carp (*Cyprinus carpio*) caused by high salinity. Biochem Cell Arch 19:4605–4610

Allain CC, Poon LS, Chan CSG, Richmond W, Fu PC (1974) Enzymatic determination of total serum cholesterol. Clin Chem 20:470–475

Alsaloom AN (2024) Antifungal, antibacterial, and antioxidant activities of camel whey protein hydrolysates. Pol J Environ Stud 33:4517–4524

Al-Shamsi KA, Mudgil P, Hassan HM, Maqsood S (2018) Camel milk protein hydrolysates with improved technofunctional properties and enhanced antioxidant potential in in vitro and in food model systems. J Dairy Sci 101:47–60

APHA (1998) Standard methods for the examination of water and wastewater, 19th edn. American Public Health Association, Washington, DC

Asztalos B, Nemcsok J (1985) Effect of pesticides on the LDH activity and isoenzyme pattern of carp (*Cyprinus carpio* L.) sera. Comp Biochem Physiol C Comp Pharmacol Toxicol 82:217–219
Baba WN, Mudgil P, Baby B, Vijayan R, Gan C-Y, Maqsood S (2021) New insights into the cholesterol esterase- and lipase-inhibiting potential of bioactive peptides from camel whey hydrolysates: identification, characterization, and molecular interaction. J Dairy Sci 104:7393–7405
Badr G, Ramadan NK, Abdel-Tawab HS, Ahmed SF, Mahmoud MH (2018) Camel whey protein protects lymphocytes from apoptosis via the PI3K–AKT, NF-κB, ATF-3, and HSP-70 signaling pathways in heat-stressed male mice. Biochem Cell Biol 96:407–416
Behrouz S, Saadat S, Memarzia A, Sarir H, Folkerts G, Boskabady MH (2022) The antioxidant, anti-inflammatory and immunomodulatory effects of camel milk. Front Immunol 13:855342
Bernet D, Schmidt H, Meier W, Burkhardt-Holm P, Wahli T (1999) Histopathology in fish: proposal for a protocol to assess aquatic pollution. J Fish Dis 22:25–34
Cheng Y, Zhao J, Ayisi CL, Cao X (2022) Effects of salinity and alkalinity on fatty acids, free amino acids and related substance anabolic metabolism of Nile tilapia. Aquac Fish 7:389–395
Chowdhury S, Saikia S (2020) Oxidative stress in fish: a review. J Sci Res 12:145–160
Dawood MA, Amer AA, Elbialy ZI, Gouda AH (2020) Effects of including triticale on growth performance, digestive enzyme activity, and growth-related genes of Nile tilapia (*Oreochromis niloticus*). Aquaculture 528:735568
Dawood MA, Noreldin AE, Sewilam H (2021) Long term salinity disrupts the hepatic function, intestinal health, and gills antioxidative status in Nile tilapia stressed with hypoxia. Ecotoxicol Environ Saf 220:112412
Doumas BT, Watson WA, Biggs HG (1971) Albumin standards and the measurement of serum albumin with bromcresol green. Clin Chim Acta 31:87–96
Du D, Lv W, Su R, Yu C, Jing X, Bai N, Hasi S (2021) Hydrolyzed camel whey protein alleviated heat stress-induced hepatocyte damage by activated Nrf2/HO-1 signaling pathway and inhibited NF-κB/NLRP3 axis. Cell Stress Chaperones 26:387–401
Elias RJ, McClements DJ, Decker EA (2005) Antioxidant activity of cysteine, tryptophan, and methionine residues in continuous phase β-lactoglobulin in oil-in-water emulsions. J Agric Food Chem 53:10248–10253
El-Sanatawy AM, Ash-Shormillesy SM, El-Yazied AA, El-Gawad HGA, Azab E, Gobouri AA, Sitohy M, Osman A (2021) Enhancing grain yield and nitrogen accumulation in wheat plants grown under a Mediterranean arid environment by foliar spray with papain-released whey peptides. Agronomy 11:1913
Fan Z, Wu D, Zhang Y, Li J, Xu Q, Wang L (2021) Carbonate alkalinity and dietary protein levels affected growth performance, intestinal immune responses and intestinal microflora in Songpu mirror carp (*Cyprinus carpio Songpu*). Aquaculture 545:737135
Fossati P, Prencipe L (1982) Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 28:2077–2080
Göçer H, Gülçin İ (2011) Caffeic acid phenethyl ester (CAPE): correlation of structure and antioxidant properties. Int J Food Sci Nutr 62:821–825
Guo M (2019) Whey protein production, chemistry, functionality, and applications. Wiley
Han CY, Zheng QM, Sun ZT (2016) Gene expression and activities of antioxidant enzymes in liver of hybrid Tilapia, *Oreochromis niloticus*× *Oreochromis aureus*, under acute pH stress. J World Aquac Soc 47:260–267
Hastuti S, Subandiyono S, Windarto S (2019) Blood performance of jaundice catfish Clarias gariepinus. Aquac Aquar Conserv Legis 12:480–489
Hoyle NT, Merritt JH (1994) Quality of fish protein hydrolysates from herring (*Clupea harengus*). J Food Sci 59:76–79
Javed M, Usmani N (2015) Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by thermal power plant effluent. Saudi J Biol Sci 22:237–242
Jiang J, Feng L, Liu Y, Jiang W-D, Hu K, Li S-H, Zhou X-Q (2013) Mechanistic target of rapamycin in common carp: cDNA cloning, characterization, and tissue expression. Gene 512:566–572
Kikuchi K, Furuta T, Iwata N, Onuki K, Noguchi T (2009) Effect of dietary lipid levels on the growth, feed utilization, body composition and blood characteristics of tiger puffer *Takifugu rubripes*. Aquaculture 298:111–117
Kilari BP, Mudgil P, Azimullah S, Bansal N, Ojha S, Maqsood S (2021) Effect of camel milk protein hydrolysates against hyperglycemia, hyperlipidemia, and associated oxidative stress in streptozotocin (STZ)-induced diabetic rats. J Dairy Sci 104:1304–1317

Kumar P, Jain K, Sardar P (2018) Effects of dietary synbiotic on innate immunity, antioxidant activity and disease resistance of Cirrhinus mrigala juveniles. Fish Shellfish Immunol 80:124–132
Li M, Wang S, Zhao Z, Luo L, Zhang R, Guo K, Zhang L, Yang Y (2022) Effects of alkalinity on the antioxidant capacity, nonspecific immune response and tissue structure of Chinese mitten crab *Eriocheir sinensis*. Fishes 7:206
Liang Q, Ou M, Li Z, Ren Y, Wei W, Qiao X, Hu R, Wu X, Liu Y, Wang W (2020) Functional analysis target of rapamycin (TOR) on the *Penaeus vannamei* in response to acute low temperature stress. Fish Shellfish Immunol 96:53–61
Liu W, Xu C, Li Z, Chen L, Wang X, Li E (2023) Reducing dietary protein content by increasing carbohydrates is more beneficial to the growth, antioxidative capacity, ion transport, and ammonia excretion of Nile tilapia (*Oreochromis niloticus*) under long-term alkalinity stress. Aquac Nut 2023:9775823
Long S, Dong X, Liu H, Yan X, Tan B, Zhang S, Chi S, Yang Q, Liu H, Yang Y (2022) Effect of dietary oxidized fish oil on liver function in hybrid grouper (♀ *Epinephelus fuscoguttatus*×♂ *Epinephelus lanceolatus*). Aquac Rep 22:101000
Maita M (2007) Fish health assessment. In: Dietary supplements for the health and quality of cultured fish. CAB International, pp 10–34. https://doi.org/10.1079/9781845931995.0010
Meyers TR (2009) Standard necropsy procedures for finfish. In: Heil H (ed) NWFHS laboratory procedures manual, 5th edn. US Fish and Wildlife Service, Washington, pp 64-74
Molina R, Moreno I, Pichardo S, Jos A, Moyano R, Monterde JG, Cameán A (2005) Acid and alkaline phosphatase activities and pathological changes induced in Tilapia fish (*Oreochromis* sp.) exposed subchronically to microcystins from toxic cyanobacterial blooms under laboratory conditions. Toxicon 46:725–735
Momen S, Salami M, Alavi F, Emam-Djomeh Z, Moosavi-Movahedi AA (2019) The techno-functional properties of camel whey protein compared to bovine whey protein for fabrication a model high protein emulsion. LWT 101:543–550
Neiffer DL, Stamper MA (2009) Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. ILAR J 50:343–360
Ngashangva L, Vinay B, Pranab G (2019) Development of new methods for determination of bilirubin. J Pharm Biomed Anal 162:272–285
NRC (2011) Nutrient requirements of fish and shrimp. National academies press
Ondrasek G, Rengel Z (2021) Environmental salinization processes: detection, implications & solutions. Sci Total Environ 754:142432
Osman A, El-Hadary A, Korish AA, AlNafea HM, Alhakbany MA, Awad AA, Abdel-Hamid M (2021a) Angiotensin-I converting enzyme inhibition and antioxidant activity of papain-hydrolyzed camel whey protein and its hepato-renal protective effects in thioacetamide-induced toxicity. Foods 10:468
Osman A, Enan G, Al-Mohammadi A-R, Abdel-Shafi S, Abdel-Hameid S, Sitohy MZ, El-Gazzar N (2021b) Antibacterial peptides produced by alcalase from cowpea seed proteins. Antibiotics 10:870
Ovie K-S, Adamu KM, Okobi IJ (2012) Sublethal effects of chromium on enzymatic activities of the African catfish: Clarias gariepinus (Burchell, 1822). Not Sci Biol 4:24–30
Oyeniran DO, Sogbanmu TO, Adesalu TA (2021) Antibiotics, algal evaluations and subacute effects of abattoir wastewater on liver function enzymes, genetic and haematologic biomarkers in the freshwater fish, Clarias gariepinus. Ecotoxicol Environ Saf 212:111982
Özok N (2020) Effects of cypermethrin on antioxidant enzymes and lipid peroxidation of Lake Van fish (*Alburnus tarichi*). Drug Chem Toxicol 43:51–56
Peres H, Santos S, Oliva-Teles A (2014) Blood chemistry profile as indicator of nutritional status in European seabass (*Dicentrarchus labrax*). Fish Physiol Biochem 40:1339–1347
Henry R (1964) Colorimetric determination of total protein. Clinical Chemistry Harper Row Publ, New York, p 181
Qu F, Xu W, Deng Z, Xie Y, Tang J, Chen Z, Luo W, Xiong D, Zhao D, Fang J (2020) Fish c-jun n-terminal kinase (Jnk) pathway is involved in bacterial mdp-induced intestinal inflammation. Front Immunol 11:459
Rahimnejad S, Dabrowski K, Izquierdo M, Malinovskyi O, Kolářová J, Policar T (2021) Effects of dietary protein and lipid levels on growth, body composition, blood biochemistry, antioxidant capacity and ammonia excretion of European grayling (*Thymallus thymallus*). Front Mar Sci 8:715636
Ramadan MF, Osman AMO, El-Akad HM (2008) Total antioxidant potential of juices and beverages-screening by DPPH in vitro assay. Dtsch Lebensm-Rundsch 104:235–239
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

Roques S, Deborde C, Richard N, Skiba-Cassy S, Moing A, Fauconneau B (2020) Metabolomics and fish nutrition: a review in the context of sustainable feed development. Rev Aquac 12:261–282
Roy PK, Rashid F, Bragg J, Ibdah JA (2008) Role of the JNK signal transduction pathway in inflammatory bowel disease. World J Gastroenterol 14:200–202
Roychowdhury P, Aftabuddin M, Pati MK (2021) Thermal stress–induced oxidative damages in the liver and associated death in fish, Labeo rohita. Fish Physiol Biochem 47:21–32
Saad AM, Osman AOM, Mohamed AS, Ramadan MF (2020) Enzymatic hydrolysis of *Phaseolus vulgaris* protein isolate: characterization of hydrolysates and effect on the quality of minced beef during cold storage. Int J Pept Res Ther 26:567–577
Salami M, Moosavi-Movahedi AA, Ehsani MR, Yousefi R, Haertle T, Chobert J-M, Razavi SH, Henrich R, Balalaie S, Ebadi SA (2010) Improvement of the antimicrobial and antioxidant activities of camel and bovine whey proteins by limited proteolysis. J Agric Food Chem 58:3297–3302
Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3:1101–1108
Shang X, Geng L, Yang J, Zhang Y, Xu W (2021) Transcriptome analysis reveals the mechanism of alkalinity exposure on spleen oxidative stress, inflammation and immune function of *Luciobarbus capito*. Ecotoxicol Environ Saf 225:112748
Shen Y, Zhang H, Zhou Y, Sun Y, Yang H, Cao Z, Qin Q, Liu C, Guo W (2021) Functional characterization of cathepsin B and its role in the antimicrobial immune responses in golden pompano (*Trachinotus ovatus*). Devel Comp Immunol 123:104128
Singh SK, Tiwari V, Chadha N, Munilkumar S, Prakash C, Pawar NA (2019) Effect of dietary synbiotic supplementation on growth, immune and physiological status of *Labeo rohita* juveniles exposed to low pH stress. Fish Shellfish Immunol 91:358–368
Song L, Zhao Y, Song Y, Zhao L, Ma C, Zhao J (2021) Effects of saline-alkaline water on growth performance, nutritional processing, and immunity in Nile tilapia (*Oreochromis niloticus*). Aquaculture 544:737036
Su H, Ma D, Zhu H, Liu Z, Gao F (2020) Transcriptomic response to three osmotic stresses in gills of hybrid tilapia (*Oreochromis mossambicus* female× *O. urolepis hornorum* male). BMC Genomics 21:1–20
Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's theory and practice of histological techniques. Elsevier health sciences, 7th edn. Churchill Livingstone. Elsevier, England
Tang J, Lu X, Chen F, Ye X, Zhou D, Yuan J, He J, Chen B, Shan X, Jiang J (2018) Effects of perfluorooctanoic acid on the associated genes expression of autophagy signaling pathway of *Carassius auratus* lymphocytes in vitro. Front Physiol 9:1748
Tanida I (2011) Autophagosome formation and molecular mechanism of autophagy. Antioxid Redox Signal 14:2201–2214
Teets NM, Peyton JT, Colinet H, Renault D, Kelley JL, Kawarasaki Y, Lee RE Jr, Denlinger DL (2012) Gene expression changes governing extreme dehydration tolerance in an Antarctic insect. Proc Natl Acad Sci 109:20744–20749
Tran-Duy A, Schrama JW, van Dam AA, Verreth JA (2008) Effects of oxygen concentration and body weight on maximum feed intake, growth and hematological parameters of Nile tilapia, *Oreochromis niloticus*. Aquaculture 275:152–162
Wang J, Sun L, Li X, Tao S, Wang F, Shi Y, Guan H, Yang Y, Zhao Z (2023) Alkali exposure induces autophagy through activation of the MAPKpathway by ROS and inhibition of mTOR in Eriocheir sinensis. Aquat Toxicol 258:106481
Wu Y, Wu T, Yang L, Su Y, Zhao C, Li L, Cai J, Dai X, Wang D, Zhou L (2023) Generation of fast growth Nile tilapia (*Oreochromis niloticus*) by myostatin gene mutation. Aquaculture 562:738762
Xia X, Wang X, Qin W, Jiang J, Cheng L (2019) Emerging regulatory mechanisms and functions of autophagy in fish. Aquaculture 511:734212
Xu J, Li Q, Xu L, Wang S, Jiang Y, Zhao Z, Zhang Y, Li J, Dong C, Xu P (2013) Gene expression changes leading extreme alkaline tolerance in Amur ide (*Leuciscus waleckii*) inhabiting soda lake. BMC Genomics 14:1–11
Xu X, Tao L, Wang A, Li L, Fan K, Shen Y, Li J (2020) Genome-wide identification of JNK and p38 gene family in Ctenopharyngodon idella and their expression profiles in response to bacterial challenge. Comp Biochem Physiol Part D Genomics Proteomics 33:100647
Yao Z, Lai Q, Zhou K, Rizalita RE, Wang H (2010) Developmental biology of medaka fish (*Oryzias latipes*) exposed to alkalinity stress. J Appl Ichthyol 26:397–402
Zhang C-N, Rahimnejad S, Lu K-L, Zhou W-H, Zhang J-L (2019) Molecular characterization of p38 MAPK from blunt snout bream (*Megalobrama amblycephala*) and its expression after ammonia stress, and lipopolysaccharide and bacterial challenge. Fish Shellfish Immunol 84:848–856

Zhang W, Belton B, Edwards P, Henriksson PJ, Little DC, Newton R, Troell M (2022) Aquaculture will continue to depend more on land than sea. Nature 603:2–4

Zhang R, Zhao Z, Li M, Luo L, Wang S, Guo K, Xu W (2023a) Metabolomics analysis reveals the response mechanism to carbonate alkalinity toxicity in the gills of Eriocheir sinensis. Comp Biochem Physiol C Toxicol Pharmacol 263:109487

Zhang Y, Wen H, Liu Y, Qi X, Sun D, Zhang C, Zhang K, Zhang M, Li J, Li Y (2023b) Gill histological and transcriptomic analysis provides insights into the response of spotted sea bass (*Lateolabrax maculatus*) to alkalinity stress. Aquaculture 563:738945

Zhao Y, Zhang C, Zhou H, Song L, Wang J, Zhao J (2020) Transcriptome changes for Nile tilapia (Oreochromis niloticus) in response to alkalinity stress. Comp Biochem Physiol Part D Genomics Proteomics 33:100651

Zhou H, Yao T, Zhang T, Sun M, Ning Z, Chen Y, Mu W (2024) Effects of chronic saline-alkaline stress on gill, liver and intestinal histology, biochemical, and immune indexes in Amur minnow (*Phoxinus lagowskii*). Aquaculture 579:740153

Authors and Affiliations

Rowida E. Ibrahim[1] · Gehad E. Elshopakey[2] · Mohamed Y. M. Aly[3] · Abdelwahab A. Abdelwarith[4] · Elsayed M. Younis[4] · Yasmina M. Abd-Elhakim[5] · Tarek Khamis[6] · Ali Osman[7] · Mohamed M. M. Metwally[8,9] · Simon J. Davies[10] · Amany Abdel-Rahman Mohamed[5]

✉ Rowida E. Ibrahim
rowidakamhawey@yahoo.com

✉ Yasmina M. Abd-Elhakim
yasmina_forensic@hotmail.com

✉ Amany Abdel-Rahman Mohamed
amanyrahman292@gmail.com

[1] Department of Aquatic Animal Medicine, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[2] Department of Clinical Pathology, Faculty of Veterinary Medicine, Mansoura University, PO Box 35516, Mansoura, Dakahlia, Egypt

[3] Pollution Laboratory, Freshwater and Lakes Division, National Institute of Oceanography and Fisheries (NIOF), Cairo, Egypt

[4] Department of Zoology, College of Science, King Saud University, PO Box 2455, 11451 Riyadh, Saudi Arabia

[5] Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Zagazig University, PO Box 44511, Zagazig, Sharkia, Egypt

[6] Department of Pharmacology, Faculty of Veterinary Medicine, Zagazig University, Zagazig 44511, Egypt

[7] Biochemistry Department, Faculty of Agriculture, Zagazig University, Zagazig 44511, Egypt

[8] Department of Pathology, Faculty of Veterinary Medicine, Zagazig University, PO Box 44511, Zagazig, Sharkia, Egypt

[9] Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, King Salman International University, Ras Sedr, Egypt

[10] Aquaculture Nutrition Research Unit ANRU, College of Science and Engineering, Carna Research Station, Ryan Institute, University of Galway, Galway H91V8Y1, Ireland