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RESEARCH

Carbonate alkalinity induces stress responses and renal and metabolic disorders in Nile tilapia: mitigation by camel whey protein hydrolysate diet

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Abstract Alkaline stress is a major concern in aquaculture that badly affects the aquatic species' health and hemostasis. This research investigated the effect of carbonate alkalinity exposure on the gills and kidney organs as important organs for hemostasis, as well as the ameliorative role of camel protein hydrolysates (CPH) as dietary additives against alkaline stress detrimental impacts in Nile tilapia (*Oreochromis niloticus*). The fish ($n=160$) were divided into four groups (G1, G2, G3, and G4), with the control (G1) fed a basal diet, while G2 was fed a basal diet supplemented with 75 g CPH/kg and was reared in freshwater (carbonate alkalinity of 1.4 µmol/L, pH = 7.19). The G3 and G4 were reared in alkaline water (carbonate alkalinity of 23.8 µmol/L, pH = 8.65) and fed the same diets as G1 and G2 for 30 days, respectively. The fish were stocked under a water temperature of 26.4 ± 1.5 °C, and the diets were introduced to the fish three times daily at a rate of 4% of their body weight. The results of this research showed that alkaline exposure increased kidney function parameters (creatinine, urea, and uric acid), glucose, and cortisol levels in the exposed fish. Alkaline exposure reduced the blood electrolytes level

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(calcium, magnesium, sodium, potassium, and chloride) and branchial antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase, and reduced glutathione) and elevated malondialdehyde level in the exposed fish. Significant downregulation of the branchial expression of Na^+/K^+ ATPase α-3 subunit (0.17-fold), calcium/calmodulin-dependant protein kinase 1 β (0.23 fold), chloride channel protein 2 (0.38-fold), solute carrier family 12 a 2 (0.33-fold), and solute carrier family 4 a 4 (0.21-fold) was in the fish-reared under carbonate alkalinity stress. Alkaline exposure induced severe histopathological changes in the gills and kidney tissue architecture including inflammatory, circulatory, degenerative, and progressive responses. Supplementation of the Nile tilapia diet with 75 g CPH/kg ameliorated renal function and balanced the blood electrolytes, glucose, and cortisol levels in the alkaline-exposed fish. Modulation of the branchial gene expression profile and improving the gills and kidney microstructure were consequences of feeding on CPH diets during alkaline stress situations. Overall, fortifying the Nile tilapia diets with 75 g CPH/kg helps the fish restore their hemostasis and metabolic status during alkaline stress exposure which enables the sustainable culture of this species in such conditions.

Keywords Nile tilapia · Gills · Kidney · Electrolytes · Alkaline stress · Gene expression

Introduction

Carbonate alkalinity is an essential measure of the water buffering ability for the growth of fish. High alkalinity aquaculture pond waters have a relatively stable pH, whereas low alkalinity waters fluctuate more. Additionally, aquatic animals are shielded from the toxicity of trace metal ions by the alkalinity (Kaushal et al. 2018). On the other hand, most fish are harmed by extremely high alkalinity, which also presents several physiological difficulties and may even lead to the degradation of cellular biomolecules (Ge et al. 2023).

In fish, alkaline stress caused gill oxidative stress through a reduction in the antioxidant enzyme activity and increased lipid peroxidation level. In addition, it induced metabolic disorders through increased blood ammonia and urea levels and altered gene expression profiles in the gill tissue (Liu et al. 2022). Fish gills are an essential organ responsible for many physiological events, such as breathing, filter-feeding, nitrogen excretion from ammonia, and osmoregulation. The function and structure of gills are influenced by alterations in various environmental factors including alkalinity level, temperature, and salinity in the surrounding water (Chen et al. 2023).

Fish kidneys have a critical role in osmoregulation (Takvam et al. 2021). Exposure to alkaline stress-induced oxidative stress altered ion balance and their regulating genes in the kidneys of Nile tilapia (*Oreochromis niloticus*) (Zhao et al. 2020). In the Crucian Carp (*Carassius auratus*), alkaline stress-induced oxidative stress caused severe damage to the kidney structure and function (Ding et al. 2023).

Dietary interventions seem to be a flexible and sustainable way to help fish develop stress resistance worldwide (Ciji and Akhtar 2021; Sherif et al. 2021; Okasha et al. 2024). Camel whey protein is an anti-stress dietary additive and has been proven to have anti-stress, anti-oxidative, and anti-inflammatory properties against heat stress in normal *Rattus norvegicus* hepatocytes (BRL-3A) cell culture (Du et al. 2021). Camel whey protein as a nutritional supplement containing biologically active protein and amino acids has been shown to have therapeutic effects (Ibrahim et al. 2018). Lactoferrin (LF), lactalbumin, lactoglobulins, lactoperoxidase, lysozyme, immunoglobulin, and other constituents are among the numerous components of camel whey protein (Badr et al. 2017). Hydrolysis of camel protein with enzymes resulted in the production of biologically active peptides that have various physiological properties (Jafar et al. 2018; Kumar et al. 2016) such as immunostimulant, anti-diabetic properties, anti-viral, anti-inflammatory, and anti-cancer (Dou et al. 2022).

The Nile tilapia (*O. niloticus*) is the third most important species of fish in aquaculture which is used widely to address the problem of food security, and its cultivation on farms has grown dramatically in several emerging countries (Geletu and Zhao 2023). Nile tilapia is widely cultivated because of its improved growth performance and the capacity to adapt to survive in various cultural conditions (Magbanua and Ragaza 2024). Nile tilapia holds significant economic and ecological value worldwide because of its euryhaline nature; it is frequently grown in brackish-water ponds (Al Jamali et al. 2023).

There are very few physiological and genetic studies on Nile tilapia's high alkalinity tolerance. In order to identify the tissue-specific transcriptome variations of *O. niloticus* at alkali stress exposure, we used biochemical assays, histopathological analysis, and RNA-Sequence technology in this investigation. A thorough analysis was conducted on the pathways and genes that react to alkaline stress and the combating impact of camel protein hydrolysates (CPH) diets. The findings offered extremely important information for comprehending the molecular mechanisms of Nile tilapia's adaptation to an alkaline environment as well as developing a new strategy for overcoming this stress situation through CPH dietary addition.

Materials and methods

Camel protein hydrolysate (CPH) preparation

The CPH preparation was described in our earlier study (Ibrahim et al. 2024) from camel milk samples (Bilbies City farm, Sharkia Governorate, Egypt). Briefly, the caseins in the milk samples were precipitated using 1 M HCl followed by centrifugation for 1 h at 6000 × g and 4 °C (Jouan C4-22 Benchtop Centrifuge, France). The obtained whey was lyophilized using 50 mM phosphate buffer and refrigerated. Enzymatic hydrolysis using trypsin enzyme (porcine pancreas, EC3.4.21.4) (Merck, KGaA, Darmstadt, Germany) under the following condition (buffer, 0.1 M Na_2HPO_4-NaH_2PO_4; pH, 8.8; temperature, 37 °C). The substrate and enzyme were mixed at an enzyme/substrate ratio of 1/200 E/S, followed by incubation for 4 h at 37 °C with continuous stirring. Boiling the mixture was done for 10 min at 100 °C. Finally, CPH was centrifuged at 5000 × g for 10 min and 4 °C; the supernatant was lyophilized and stored at − 20 °C till required. The degree of hydrolysis (DH) was assessed utilizing the trichloroacetic acid (TCA) method following the protocol described by Hoyle and Merritt (1994). Briefly, 20 mL of CPH was mixed with 20 mL of TCA (20%; w/v), then the mixture was left to stand for 30 min, followed by centrifugation at 8000 × g for 10 min. The protein level in the supernatant was measured following the Kjeldahl protocol; finally, the DH (%) was calculated as follows:

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

Fish and conditions of raising

Cultured *O. niloticus* were obtained from the Fish Research Unit (Zagazig University, Egypt). The fish underwent a health examination and were given 2 weeks to adjust to the laboratory settings before the trial began and stocked as 10 fish per 100-L aquarium. The aquaria were supplied with free-chlorine water and a fluorescent tube to maintain a 12-h light: 12-h dark period. By the APHA guidelines (2005), the water criteria measurements included descriptions as follows: water temperature (26.4 ± 1.5 °C), dissolved oxygen (6.8 ± 0.4 mg/L), ammonia (0.02 ± 0.001 mg/L), and pH (7.10 ± 0.9).

Diet preparation

The Zagazig University Authority for Animal Use in Research checked out and authorized the experimental protocol (ZU-IACUC/2/F/393/2023). According to NRC (2011), two diets were created to satisfy Nile tilapia's dietary requirements (Table 1). The control diet was a basal diet without any additions, while the CPH diet was a basal diet fortified with CPH at a level of 75 g kg^{-1}. The CPH dietary level of 75 g kg^{-1} was selected according to the protocol of Ibrahim et al. (2024).

Experimental design

Nile tilapia, *Orochromis niloticus* ($n = 160$; average weight = 16.02 ± 0.14 g), were stocked in 16 glass aquariums with a total water capacity of 100 L per each and were divided into four groups, with four replicates for each (40/group; 10/replicate). The first group (C) was fed on the basal control diet and the second group (CP) was fed on 75 g of CPH per kg of basal diet, respectively, and raised in freshwater with carbonate alkalinity of 1.4 mmol/L (pH = 7.19). The basal diet and the CPH diet were given to the third (AL) and fourth (AL + CP) groups, respectively, and they were raised in alkaline conditions with carbonate alkalinity of 23.8 mmol/L (pH = 8.65) (Cheng et al. 2022). Throughout the study period, the diets were offered three times a day (10 a.m., 1 p.m., and 4

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

Ingredients	Basal
Fish meal	180
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	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

[#]Premix (AQUA-MIN®, Plexopharm, Egypt)

[*]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

p.m.) up to satisfaction at a rate of 4% of their body weight. Records were kept of fish deaths and clinical indicators.

Sampling

Fish were put to sleep with a 100 mg/L benzocaine solution at the end of the study (Neiffer and Stamper 2009) before blood samples (12 fish/ group) were taken from the caudal vessels. The serum was separated from the blood at 3000 rpm (for 10 min at 4 °C) and then kept at − 20 °C till biochemical assays were conducted. Gills and kidney tissues (12/group) were sampled and stored in 10% neutral buffered formalin for histological analysis. Gills tissue (12/group) was sampled for oxidant/antioxidant and gene expression assay evaluation. The samples for gene expression analysis were preserved in 1 mL of QIAzol reagent (Qiagen, Germany), and they were frozen at − 80 °C.

Branchial oxidant/antioxidant status evaluation

The gill tissues were subjected to a 3-time washing process using a cold NaCl solution (0.9%) followed by homogenization in PBS (pH 7.5). Afterward, cold centrifugation for 15 min at 3000 × *g* was done on the homogenates, which were cold centrifuged. The supernatants were meticulously taken in a clean tube to measure antioxidants and oxidative stress indicators (Ibrahim et al. 2022).

The oxidant/antioxidant barometers including malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and reduced glutathione (GSH) were evaluated spectrophotometrically in the gills homogenates following the kits obtained from the Bio-Diagnostic Company (Cairo, Egypt). Following the kit (catalog No. MD2529), MDA was determined by the thiobarbituric acid (TBA) technique. Briefly, TBA reacts with MDA to produce TBA reactive product which was measured at 534 nm. Following the kit (catalog No. SD2521), SOD was measured. The SOD suppresses superoxide anion free radical (O^{2-}) which in turn decreases the formation of nitrite; the sample was measured at an absorbance of 550 nm. The CAT (catalog No. CA2517) was measured based on the enzymatic reaction between samples, hydrogen peroxide (H_2O_2), and potassium phosphate (pH 7.0). The amount of enzyme that breaks down H_2O_2 per min in samples was used to measure CAT activity at an absorbance of 240 nm. GPx (catalog No. GP2524) was measured based on the oxidation of NADPH (nicotinamide adenine dinucleotide phosphate) to $NADP^+$. The samples were measured at an absorbance of 340 nm. GSH (catalog No. TA2511) was measured based on the reduction of 5,5′dithiobis (2-nitrobenzoic acid, DTNB) by the GSH till it gets a yellow color, which was measured at an absorbance of 405 nm.

Biochemical indices

The creatinine level was determined using the Fossati et al. (1983) protocol. The levels of serum uric acid (Spinreact Co., Santa Coloma, Spain, Catalog No.: MD41001) and urea (MyBioSource Co., California, USA, Catalog No.: MBS9374784) were measured in compliance with manufacturer instructions. Using the techniques of Saliu et al. (2017) and Trinder

(1969), the cortisol and glucose levels in the serum were measured by calorimetric analysis. Serum concentrations of calcium (Ca) and magnesium (Mg) were measured using DiaSys (Diagnostic Systems GmbH, Holzheim, Germany) commercial kits and the semi-automated clinical chemistry analyzer Randox RX Monza (Randox Laboratories, Crumlin, UK) (Kovacik et al. 2017). Sodium (Na), potassium (K), and chloride (Cl) ions were analyzed using an Easy-Lite analyzer (Medica, Bedford, MA, USA) provided with an ion-selective electrode (Massanyi et al. 2014; Kolesarova et al. 2008).

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

Extraction of the total RNA was carried out from frozen gills specimens using the QIAzol reagent. Using the Quantitect® Reverse Transcription kit (Qiagen, Germany) instructions, the extracted RNA was used to transcribe the first strand of cDNA. The forward and reverse sequences of the primers for the genes are shown in Table 2, where the housekeeping gene (ubiquitin-conjugating enzyme, *ubce2*) is included. The QuantiTect® SYBR® Green PCR kit (Qiagen, Germany) was utilized for the qPCR analysis, which was conducted in a Rotor-Gene Q instrument with 40 cycles of 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. The comparative $2^{-\Delta\Delta Ct}$ method was utilized to calculate the relative mRNA expression pattern of each gene (Schmittgen and Livak 2008).

Histopathological analysis

Gills and kidney samples were extracted, quickly fixed in 10% neutral buffered formalin, dried in progressively stronger alcohol solutions, cleaned in xylene, and finally embedded in melted paraffin wax. Using a Leica® microtome, thin (5 µm) paraffin slices were cut, and these sections were then stained with hematoxylin and eosin for microscopic examination (Suvarna et al. 2018). The stained slides were examined, and any modifications to the histology were recorded using the AmScope ToupView v4.8.15934 software (AmScope, Irvine, CA, USA).

Statistical analysis

The Shapiro–Wilk test was performed to ensure that the results were regularly distributed. Using IBM's SPSS version 17, a one-way analysis of variance (ANOVA) was used to statistically analyze the experimental results. Tukey's multiple comparisons post hoc test was utilized to compare the means of different groups ($n = 12$/group), and the statistical significance was deemed acceptable at $P < 0.05$. The analysis's findings were displayed as means ± standard error (SE).

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			
ATP-1a3	TCAGCTGAAGGGGGCATTTT	59.89	100.5	118	XM_005459087.4
	TTCAGTCGGCTCTCCTCAGA	59.96			
CAMK1β	GCTTCACTGTGTCCGTCCTT	60.25	99.61	195	XM_003441376.5
	TCCAGCGGCATCTTCTTGTT	59.96			
CLCN2	CCTGCTCTGCACACACAAAC	59.97	95	126	XM_003456312.5
	CAGGCAAGCTGTAATGGGGA	60.03			
SLC12a2	GAAGCTGGGATAGGACGCTC	59.97	102.39	129	XM_003439377.5
	CCAGGCGAATGCCATCATTG	59.97			
SLC4a4	GAAGGCCGAACTGAAGGACA	59.97	96.35	135	XM_003444476.5
	GGGGTGGAAAACAGCCTACT	59.6			

Tm melting temperature; *ubce2* ubiquitin-conjugating enzyme; *ATP-1a3* Na^+/K^+ ATPase α−3 subunit, *CAMK1β* calcium/calmodulin-dependant protein kinase 1 β, *CLCN2* chloride channel protein 2, *SLC12a2* solute carrier family 12 a 2, *SLC4a4* solute carrier family 4 a 4

Results

Oxidant/antioxidant parameters

The CP group exhibited significantly lower levels of MDA with significantly higher ($P < 0.001$) levels of antioxidant enzyme activity (SOD, CAT, GPx, and GSH) in comparison to the C group (Table 3). Compared to the C group, the branchial MDA level was significantly higher ($P < 0.001$) with lower antioxidant enzyme activity in the AL group, while these indices were modulated in the AL+CP group when compared to the AL group.

Kidney functions and stress parameters

Creatinine, urea, and uric acid levels exhibited no considerable difference between the C and CP groups. These parameters were considerably higher ($P < 0.001$) in the AL group comparable to the C group, while modulated in the AL + CP group when compared to the AL group (Table 4). The CP group showed a substantial decline in the level of blood cortisol comparable to the C group, while blood glucose levels did not differ between the CP and C groups (Table 4). The blood glucose and cortisol were considerably higher ($P < 0.001$) in the AL group comparable to the C group, while their levels were lowered in the AL + CP group when compared to the AL group.

Table 3 Oxidant/antioxidant parameters of Nile tilapia reared in alkaline stress and fed on camel protein hydrolysates (CP) diet for 30 days

	C	CP	AL	AL + CP	*P*-value
MDA (nmol mg^{-1})	1.10 ± 0.06^{c}	0.75 ± 0.25^{d}	5.67 ± 0.07^{a}	2.26 ± 0.12^{b}	< 0.001
SOD (U mg^{-1})	243.12 ± 3.09^{b}	410.83 ± 0.67^{a}	136.36 ± 2.42^{d}	174.17 ± 3.38^{c}	< 0.001
CAT (U mg^{-1})	4.88 ± 0.65^{b}	7.97 ± 0.23^{a}	0.57 ± 0.05^{d}	3.51 ± 0.21^{c}	< 0.001
GPx (U mg^{-1})	193.13 ± 2.67^{b}	288.73 ± 1.94^{a}	130.85 ± 3.08^{d}	162.37 ± 6.95^{c}	< 0.001
GSH (U mg^{-1})	171.76 ± 2.45^{b}	214.15 ± 2.57^{a}	24.38 ± 5.39^{d}	142.32 ± 2.79^{c}	< 0.001

C and CP groups were fed on 0 and 75 g of CPH per kg of basal diets and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diets and reared in alkaline water

MDA malondialdehyde, *SOD* superoxide dismutase, *CAT* catalase, *GPx* glutathione peroxidase, *GSH* reduced glutathione

Table 4 Effect of dietary supplementation of camel protein hydrolysates (CP) on the biochemical indices of Nile tilapia exposed to alkaline stress for 30 days

Parameters	C	CP	AL	AL + CP	*P*-value
Creatinine (mg dL^{-1})	0.50 ± 0.01^{b}	0.48 ± 0.01^{b}	0.79 ± 0.02^{a}	0.75 ± 0.01^{a}	< 0.001
Urea (mg dL^{-1})	19.93 ± 0.63^{b}	19.48 ± 0.60^{b}	27.79 ± 1.61^{a}	25.48 ± 0.34^{a}	< 0.001
Uric acid (mg dL^{-1})	3.69 ± 0.10^{b}	3.90 ± 0.07^{b}	4.83 ± 0.03^{a}	4.84 ± 0.05^{a}	< 0.001
Glucose (mg dL^−1)	114.39 ± 4.33^{c}	104.04 ± 3.50^{c}	199.09 ± 1.10^{a}	147.39 ± 4.07^{b}	< 0.001
Cortisol (ng mL^{-1})	22.81 ± 0.93^{d}	16.32 ± 1.48^{c}	44.23 ± 2.33^{a}	37.05 ± 1.83^{b}	< 0.001
Ca (mg dL^{-1})	9.81 ± 0.02^{a}	9.44 ± 0.05^{a}	6.75 ± 0.03^{c}	8.29 ± 0.02^{b}	< 0.001
Mg (mg dL^{-1})	5.33 ± 0.04^{a}	5.42 ± 0.01^{a}	2.42 ± 0.04^{c}	3.92 ± 0.04^{b}	< 0.001
Na (mmol L^{-1})	149.78 ± 0.25^{a}	149.01 ± 0.87^{a}	131.71 ± 0.30^{c}	141.68 ± 0.40^{b}	0.004
K (mmol L^{-1})	4.38 ± 0.09^{a}	4.05 ± 0.09^{a}	2.13 ± 0.03^{b}	3.01 ± 0.06^{b}	0.03
Cl (mmol L^{-1})	94.46 ± 1.27^{a}	92.76 ± 1.63^{a}	68.70 ± 2.93^{c}	88.07 ± 1.83^{b}	< 0.001

Variation in the data was expressed as means ± *SE*. Ca, calcium; Mg, magnesium; Na, sodium; K, potassium; Cl, chloride. C and CP groups were fed on basal diets supplemented with 0 and 75 g of CPH per kg of basal diet and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diet and reared in alkaline water

[a,b,c]Mean values in the same row with different superscripts differ significantly ($P < 0.05$)

Serum ion level

There was no substantial change between the C and CP groups in the ions level (Ca, Mg, Na, K, and Cl) (Table 3). A considerable decrease ($P < 0.001$ for Ca, Mg, and Cl; $P = 0.004$ for Na, and $P = 0.03$ for Cl) in the serum ion levels in the AL group comparable to the C group. These levels were considerably restored in the AL + CP group when compared to the AL group but maintained lower than the control level.

Gene expression results

Compared to the C group, the CP group exhibited no considerable change in the gills mRNA expression of *ATP-1a3* and *CLCN-2*, while showing a considerable rise in the expression of *CAMK-1β* (Fig. 1A). The expression of *ATP-1a3*, *CAMK-1β*, and *CLCN-2* was considerably downregulated ($P < 0.05$) in the AL group comparable to the C group. The expression of these genes was ameliorated in the AL + CP group comparable to the AL one. Comparable to the C group, the expression was 1.04-, 0.17-, and 0.71-fold for *ATP-1a3*; 1.11-, 0.23-, and 0.82-fold for *CAMK-1β*; and 1.15-, 0.38-, and 0.88-fold for *CLCN-2* in the CP, AL, and AL + CP groups, respectively.

Compared to the C group, the mRNA expression of *SLC12a2* was increased in the CP group. The expression of *SLC4a4* did not differ between the C and CP groups (Fig. 1B). In comparison to the C group, the expression of *SLC12a2* and *SLC4a4* was considerably decreased ($P < 0.05$) in the AL group, while modulated in the AL + CP group when compared to the AL group. In comparison to the C group, the expression was 1.26-, 0.33-, and 0.80-fold for *SLC12a2* and 1.05-, 0.21-, and 0.78-fold for *SLC4a4* in the CP, AL, and AL + CP groups, respectively.

Histopathological results

Normal histology was seen upon the microscopical examination of the branchial tissue sections of the fish in the C and CP groups (Fig. 2A and B). The branchial tissues of the AL group were significantly affected by the alkaline water stress. This was evidenced by numerous histological alterations including inflammatory response (lamellar inflammatory cell infiltrations particularly the lymphocytes and

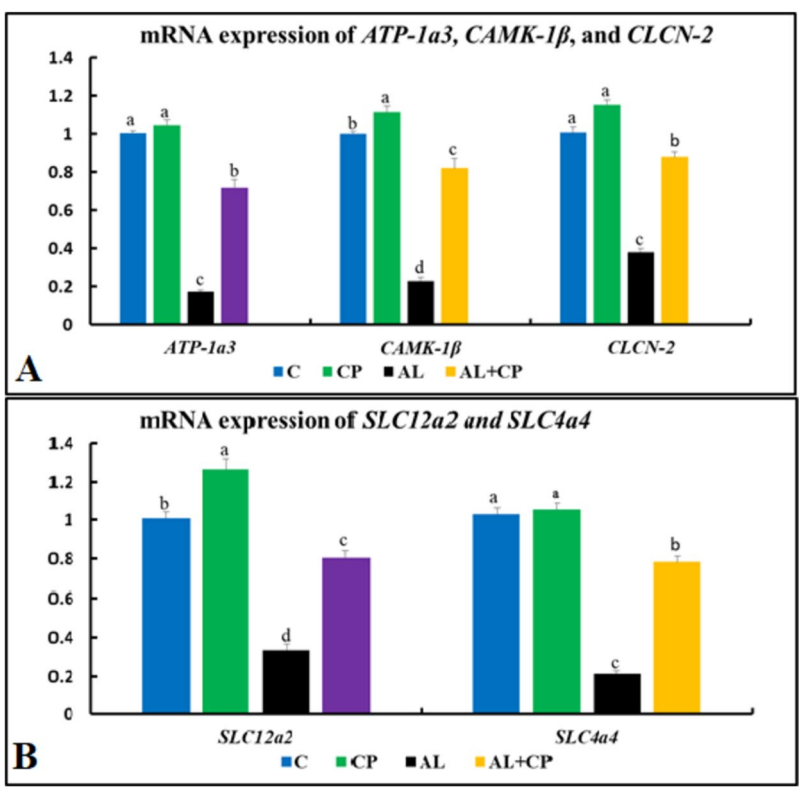

Fig. 1 mRNA expression of Na^+/K^+ ATPase α−3 subunit (*ATP-1a3*), calcium/calmodulin-dependant protein kinase-1 β (*CAMK-1β*), chloride channel protein-2 (*CLCN-2*) (**A**), solute carrier family 12 a 2 (*SLC12a2*) and solute carrier family 4 a 4 (*SLC4a4*) (**B**) of Nile tilapia exposed to carbonate alkalinity and fed camel protein hydrolysates (CPH) for 30 days. C and CP groups were fed on 0 and 75 g of CPH per kg of basal diet and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diet and reared in alkaline water

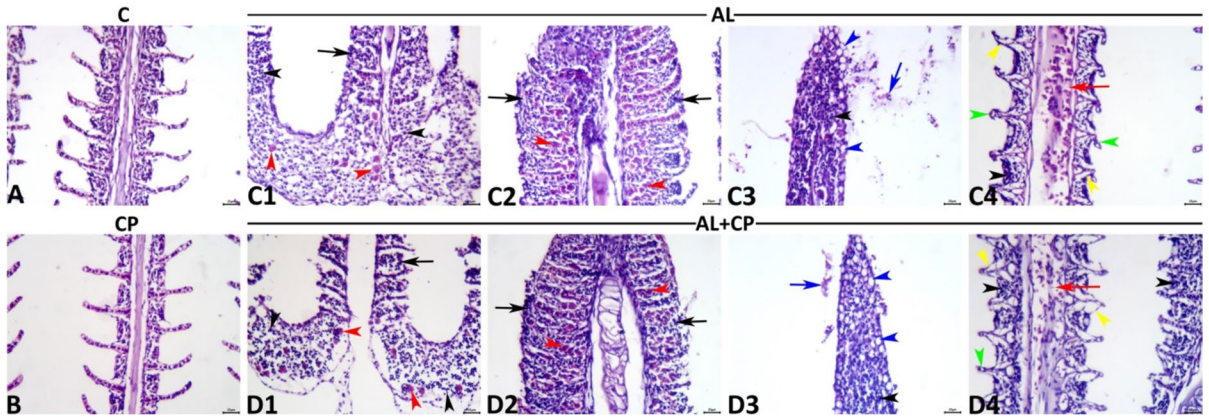

Fig. 2 Representative H&E-stained light photomicrographs of the gill tissue sections showing normal histological picture in the C (**A**) and the CP (**B**) groups. The gill tissue sections of the AL group (**C1**, **C2**, **C3**, and **C4**) show alkaline stress histological alterations; lamellar inflammatory cell infiltrations particularly the lymphocytes (black arrowheads) and the eosinophilic granular cells (red arrowheads), vascular congestion (red arrows), lamellar shorting with or without bending (green arrowheads), and epithelial lifting (yellow arrowheads), epithelial desquamation, and excess mucous (blue arrows), goblet cell hyperplasia (blue arrowheads), and lamellar epithelium hyperplasia with fusion of secondary lamellae (black arrows). The gill tissue sections of the AL + CP group (**D1**, **D2**, **D3**, and **D4**) show slightly milder lesions. The scale bar equals 25 microns. C and CP groups were fed on 0 and 75 g of CPH per kg of basal diet and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diet and reared in alkaline water

the eosinophilic granular cells), circulatory response (vascular congestion, capillary channel dilation, and minute hemorrhages), degenerative response (lamellar bending, shortening, sloughing, and epithelial lifting, desquamation, and necrosis of the lamellar epithelium), and progressive response (goblet cell hyperplasia with excess mucous secretion, and lamellar epithelium hyperplasia with or without fusion of secondary lamellae) (Fig. 2C1, C2, C3 and C4). Although similar lesions were observed in both AL and AL + CP groups, the severity was somewhat milder in the latter group (Fig. 2D1, D2, D3, and D4). The encountered histological alterations in the gill tissue sections and the gill indices of all groups were presented in Table 5.

Posterior kidney

Normal histological architectures with no pathological alterations were seen in the renal tissue sections of the C and CP groups (Fig. 3A and B). The effect of alkaline water stress on the renal tissues of the AL was obvious as numerous nephropathic changes were observed in most tissue sections of that group. The nature of these changes was inflammatory (associated with tubular calcification) and interstitial tissue (interstitial mononuclear inflammatory cell infiltrates), circulatory (interstitial edema, hemorrhages, and congestion), regressive (glomerular collapse, and necrosis, with tubular vacuolation, pyknosis, necrosis), and progressive (hyperplasia of the melanomacrophage aggregates) (Fig. 3C1, C2, C3, and C4). The CPH supplementation showed average nephroprotective effects against the alkaline water stress-induced nephropathic histological alterations in the AL + CP group. This was evidenced histologically by the modest reduction in both the severities, and frequencies of the renal lesions in the AL + CP group, besides numerous regenerated tubular epithelia indicated by the tubular basophilia in numerous tissue sections (Fig. 3D1, D2, D3, and D4). The histological changes in the kidney tissue sections and the kidney indices of all groups are presented in Table 5.

Discussion

Carbonate alkalinity stress is a significant risk to fish that live in alkaline water (Yao et al. 2010). In freshwater fish, the gills and kidneys are important organs that play a crucial role in fish physiology by regulating acid–base and osmotic balance (Copatti

Table 5 Effect of CP or alkaline water stress either individually or in combination on the histology of the branchial, renal tissues and the gill, and kidney indices of *Oreochromis niloticus*

Organ	Lesions			C		CP		AL		AL + CP	
	Reaction pattern	Histological alteration	*W*	*F*	Index	*F*	Index	*F*	Index	*F*	Index
Gills	Inflammatory alterations	Leukocytic infiltration	2	0	0	0	0	89	2.60 ± 0.52[a]	62	1.40 ± 0.42[b]
	Circulatory alterations	Congestion	1	0	0	0	0	71	1.10 ± 0.31[a]	54	0.60 ± 0.22[b]
		Hemorrhages	2	0	0	0	0	12	0.20 ± 0.13	9	0.10 ± 0.10
	Regressive alterations	Epithelial lifting	1	0	0	0	0	92	2 ± 0.36[a]	82	1.50 ± 0.34[a]
		Epithelial desquamation	2	0	0	0	0	32	0.80 ± 0.32	27	0.60 ± 0.30
		Epithelial necrosis	3	0	0	0	0	36	1.20 ± 0.48	24	0.60 ± 0.40
		Lamellar shortening	2	0	0	0	0	21	0.60 ± 0.30	17	0.60 ± 0.30
		Lamellar sloughing	3	0	0	0	0	11	0.30 ± 0.30	9	0.30 ± 0.30
		Lamellar bending	2	0	0	0	0	24	0.60 ± 0.30	21	0.40 ± 0.26
	Progressive alterations	Epithelial hyperplasia	2	0	0	0	0	76	1.60 ± 0.26[a]	67	1.40 ± 0.30[a]
		Goblet cell hyperplasia	2	0	0	0	0	82	1.60 ± 0.26[a]	71	1.40 ± 0.30[a]
		Lamellar fusion	2	0	0	0	0	54	1 ± 0.33[a]	36	0.80 ± 0.32[b]
	Neoplastic alterations	Adenomas, carcinomas and/or sarcomas	3	0	0	0	0	0	1.04	0	0.74
Gill index				0		0					
Kidney	Inflammatory alterations	Leukocytic infiltration	2	0	0	0	0	36	0.80 ± 0.32	21	0.40 ± 0.26
	Circulatory alterations	Congestion	1	0	0	0	0	42	0.50 ± 0.22	34	0.30 ± 0.15
		Edema	1	0	0	0	0	29	0.30 ± 0.15	18	0.20 ± 0.12
		Hemorrhage	1	0	0	0	0	14	0.20 ± 0.20	12	0.10 ± 0.10
	Regressive alterations	Glomerular collapse	2	0	0	0	0	29	0.60 ± 0.30	21	0.20 ± 0.13
		Glomerular necrosis	3	0	0	0	0	19	0.60 ± 0.40	7	0.30 ± 0.30
		Tubular vacuolation	1	0	0	0	0	84	0.80 ± 0.13[a]	51	0.50 ± 0.16[a]
		Tubular necrosis	3	0	0	0	0	16	0.60 ± 0.40	9	0.30 ± 0.30
		Cast formations	1	0	0	0	0	4	0.10 ± 0.10	0	0
	Progressive alterations	Tubular basophilia	2	0	0	0	0	3	0.10 ± 0.10	19	0.40 ± 0.26
		melanomacrophage aggregates hyperplasia	2	0	0	0	0	41	0.80 ± 0.32[a]	49	1 ± 0.33[a]
	Neoplastic alterations	Adenomas and/or carcinomas	3	0	0	0	0	0	0	0	0
Kidney index				0		0		0.45		0.28	

Values are represented as the mean ± SE. The means within the same row carrying different superscripts are significant at $P < 0.05$. C and CP groups were fed on basal diets supplemented with 0 and 75 g of CPH per kg of basal diet and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diet and reared in alkaline water

W important factor, *F* frequency

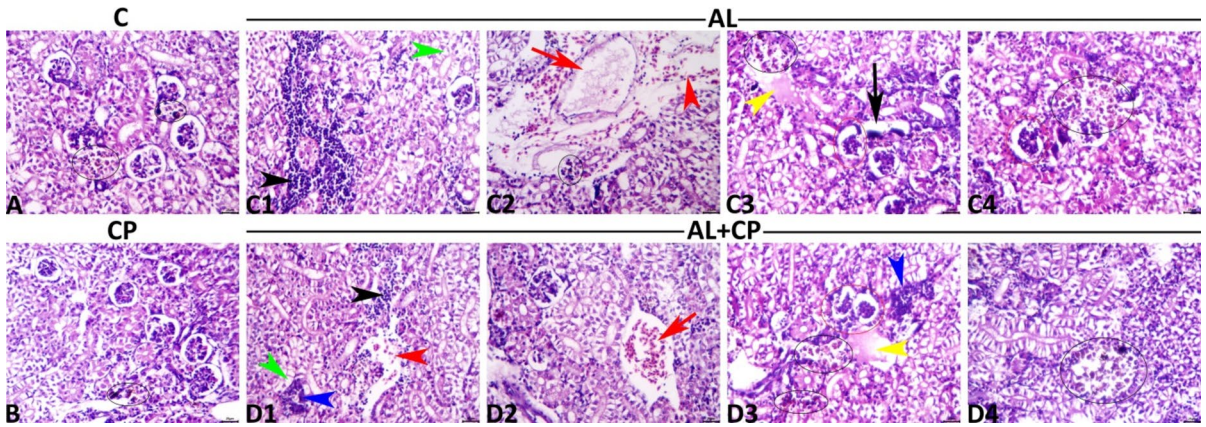

Fig. 3 Representative H&E-stained light photomicrographs of the kidney tissue sections showing normal histological picture in the C (**A**) and the CP (**B**) groups. The kidney tissue sections of the AL group (**C1**, **C2**, **C3**, and **C4**) show alkaline stress histological alterations; interstitial lymphocytic infiltration (black arrowheads), tubular vacuolation (green arrowheads), vascular congestion (red arrows), interstitial hemorrhage (red arrowheads), interstitial edema (yellow arrowheads), glomerular collapse (red ellipses), glomerular necrosis (black arrow), and hyperplasia of the melanomacrophage aggregates (black ellipses). The kidney tissue sections of the AL + CP group (**D1**, **D2**, **D3**, and **D4**) show slightly milder lesions, besides tubular basophilia (blue arrowheads). The scale bar equals 25 microns. C and CP groups were fed on 0 and 75 g of CPH per kg of basal diet and reared in fresh water. AL and AL + CP were fish groups fed on 0 and 75 g of CPH per kg of basal diet and reared in alkaline water

et al. 2019). In aquatic animals, gills are crucial to their reaction to alkaline stress (Ge et al. 2023). In addition, the kidney's primary role in freshwater teleosts is to dilute urine, which makes it possible for the extra body water that has entered through osmosis through the gills to be expelled (Bolner and Baldisserotto 2007). Our research investigated the microstructure and functions of the main osmoregulatory organs (gills and kidneys) associated with alkaline exposure in fish. As well as providing a feeding strategy (CPH at 75 g/kg diet) for mitigating the carbonate alkalinity exposure.

Fish have evolved a variety of defense mechanisms to protect themselves from oxidative stress brought on by environmental stressors (Wen et al. 2018). When an organism is in normal physiological conditions, the antioxidant system is in charge of preserving the homeostasis of reactive oxygen species (ROS) (Birnie-Gauvin et al. 2017; Kim et al. 2014). However, excessive ROS accumulation happens when ROS production surpasses the antioxidant system's capacity or when the ROS detoxifying system is weak (Bal et al. 2021; Shin et al. 2010), which induces lipid peroxidation, severely damages the cell membrane's structure and function, and ultimately results in cell death (Lushchak 2011). Superoxide radicals are normally neutralized to H_2O_2 and then to water by the main front-line antioxidant enzymes, such as SOD, CAT, GPx, and GSH (Bal et al. 2021; Sherif et al. 2024). One important metric to assess the level of tissue damage and lipid peroxidation brought on by ROS is the MDA content (Katikaneni et al. 2020). Our research revealed that the Nile tilapia exposed to alkaline stress exhibited an oxidative stress condition evident by higher MDA levels and lower antioxidant enzymes (SOD, CAT, GPx, and GSH). Numerous earlier studies have shown that exposure to alkaline environments causes an excessive build-up of ROS and oxidative stress in fish (Jiang et al. 2022). Such oxidative stress induced by alkaline exposure could be a result of rises in energy metabolism in fish in order to preserve the osmotic balance (Gan et al. 2016). This may lead to increased levels of oxidation and the accumulation of ROS. Interestingly, feeding on the CPH diet during alkaline stress enhanced the activity of antioxidant enzymes and lowered the MDA level. These results could attributed to the antioxidant properties of CPH (Ibrahim et al. 2024) due to its content of smaller peptide fractions which contain biologically active amino acids (Osman et al. 2021). These amino acids displayed higher antioxidant activity (Salami et al. 2011; Addar et al. 2019).

In addition, the CPH contains cysteine, tyrosine, phenylalanine, histidine, and tryptophan in their structure; these amino acids can scavenge the ROS (Elias et al. 2005).

Creatinine, urea, and uric acid are critical indicators of renal function (Li et al. 2021; Nwizugbo et al. 2023). The results of our study showed that the kidney function parameters (creatinine, urea, and uric acid) were elevated due to alkalinity exposure. These findings were previously reported in *C. auratus* exposed to high alkalinity stress (Ding et al. 2023). These findings may be related to the harm that carbonate alkaline stress causes to renal tubules and glomeruli. Aquatic animals may experience alterations in their tissue morphology as a result of oxidative stress brought on by environmental stressors, which will ultimately result in functional changes (Zhang et al. 2023; Huang et al. 2022). Histopathology in the current study demonstrated the alterations in the renal tissue of Nile tilapia exposed to alkaline stress. In this study, the kidney of alkaline-exposed fish showed various alterations including inflammatory (associated with tubular calcification) and interstitial tissue (interstitial mononuclear inflammatory cell infiltrates), circulatory (interstitial edema, hemorrhages, and congestion), regressive (glomerular collapse, and necrosis, with tubular vacuolation, pyknosis, necrosis), and progressive (hyperplasia of the melanomacrophage aggregates). These pathological alterations could be attributed to high ROS levels as a result of alkali stress exposure, which in turn could impair the tissue functions and structure (Lushchak 2011; Ding et al. 2023). The result of our research found that feeding the alkaline exposed fish on CPH diets modulated the renal function barometers and structure. These findings could attributed to the tissue-protective effect of the CPH which was previously proven in the renal tissue of rats after thioacetamide toxicity (Osman et al. 2021). The bioactive peptides in CPH have strong antioxidant qualities and ROS-scavenging abilities that prevent the renal tissue from further suffering from oxidative stress-related damage from alkaline exposure.

In this study, the glucose and cortisol levels were elevated in the alkaline-exposed fish. Similar findings were reported in *C. auratus* due to alkaline exposure (Ding et al. 2023). When fish are exposed to alkaline conditions, cortisol can be released quickly into their bloodstream (da Paixao Lemos et al. 2018) which is linked to ion regulation and the mobilization of energy substrates (Zhou et al. 2024). Elevated glucose levels could be a sequence for elevated cortisol levels to meet the high energy demand for competing the stress condition (Zhou et al. 2024). In this study, feeding on the CPH diet lowered the glucose and cortisol levels in the alkaline-exposed fish. CPH was reported to have a glucose-reducing ability which helps to maintain glucose homeostasis (Izadi et al. 2019).

The results of our research found that under alkaline stress conditions, the blood electrolyte (Ca, Mg, Na, K, and Cl) levels of Nile tilapia were reduced. Similar findings were reported by Wilkie and Wood (1996) who stated that alkaline water temporarily reduces the ion transport system's capacity directly affecting the Cl and Na transport sites in the gills. In our study, the electrolyte transport was closely related to the branchial expression of *ATP-1a3*, *CAMK1-β*, *CLCN-2*, *SLC12a2*, and *SLC4a4*. The expression of these genes was considerably downregulated under alkaline stress conditions. *ATP-1a3* is primarily responsible for preserving the cytoplasmic membrane's ion permeability and the equilibrium of osmotic pressure between the intracellular and external environments (Geng et al. 2016). An essential transducer of Ca^{2+} signals, *CAMK1-β* coordinates various cellular processes in fish (Churcher et al. 2014). *CLCN-2* is important for transepithelial Cl^- transport in the gills of fish (Tang and Lee 2007). *SLC12a2* is the $Na^+/K^+/2Cl^-$ symporter, which regulates the ions transport in the fish gills (Marx et al. 2022). *SLC4a4* is a member of the SLC4 family, which are integral membrane proteins that transport electrolytes such as Na^+ and Cl^-, as well as bicarbonate and/or carbonate, across the plasma membrane (Romero 2005). In this study, the downregulation of the abovementioned genes resulted in a decrease in the electrolyte level in the fish blood. These consequences could be attributed to the histopathological alterations induced as a result of alkaline stress exposure. In this study, the branchial tissue of the alkaline-exposed fish showed inflammatory, circulatory, degenerative, and progressive responses. Similar histopathological alterations were observed in the gills of shrimp (*Exopalaemon carinicauda*) (Ge et al. 2023) and Rohu (*Labeo rohita*) (Acharya et al. 2005) as a result of oxidative damage caused by alkaline exposure. By feeding on the CPH diet, the blood electrolyte levels were enhanced toward the control

level. Besides, the branchial gene expression was modulated which helped the fish under alkaline stress to conserve their normal hemostasis. Antioxidant defense systems and the activation of enzymatic antioxidants (SOD, CAT, GPx, and GSH) through ROS scavenging may be responsible for these results. This could improve tissue integrity and function (Hamed et al. 2019).

Conclusion

Exposure to carbonate alkalinity disrupted the hemostasis and induced oxidative stress and metabolic disorders in Nile tilapia. Fish exposed to alkali conditions showed elevated kidney function parameters with higher glucose and cortisol levels. The blood electrolyte level was deviated from the normal condition due to alkali stress. Alkaline stress conditions induced severe histopathological alterations in both gills and kidney tissues. The ion-related gene expression of alkaline-exposed fish was downregulated. Fortifying the Nile tilapia diet with a 75 g CPH/kg basal diet restored the hemostasis and the metabolism of fish toward normal conditions during alkaline exposure. Amelioration of the gills and kidney microstructure and functions was a consequence of feeding on the CPH diet. CPH supplementation to the Nile tilapia diet modulated the transcriptomic profile of the fish under an alkali stress situation. Consequently, our findings offer insight and understanding of the cellular and molecular processes in gills and kidneys underlying the detrimental impacts of alkaline water in Nile tilapia, as well as the adaptation mechanisms to such alkaline stress triggered by CPH dietary intervention.

Author contribution R. E.I., A.A.A., E.M.Y., A.A.M., T. K., A.O., M. M. M. M., S.J. D., and Y.M. A: conceptualization, data curation, formal analysis, investigation, methodology, resources, validation, visualization, and writing review and editing. REI: Writing original draft. All authors read, reviewed, and approved the final manuscript.

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Data availability No datasets were generated or analysed during the current study.

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 review and approve the manuscript for publication.

Conflict of interest The authors declare no competing interests.

References

Acharya S, Dutta T, Das MK (2005) Physiological and ultrastructural changes in Labeo rohita (Hamilton-Buchanan) fingerlings exposed to sublethal acidic and alkaline pH for long duration. Asian Fish Sci 18(3/4):295

Addar L, Bensouici C, Zennia SSA, Haroun SB, Mati A (2019) Antioxidant, tyrosinase and urease inhibitory activities of camel αS-casein and its hydrolysate fractions. Small Rumin Res 173:30–35

Al Jamali AH (2023) Re-evaluating eurahaline nature of Nile tilapia, *Oreochromisniloticus*: a hatchery perspective. Int J Fish Aquat Stud 11:223–231

APHA (2005) Standard methods for examination of water and wastewater, 21st edn. American Public Health Association, American Water Works Association, Water Pollution Control Federation, New York, pp 1–8

Badr G, Ramadan NK, Sayed LH, Badr BM, Omar HM, Selamoglu Z (2017) Why whey? Camel whey protein as a new dietary approach to the management of free radicals and for the treatment of different health disorders. Iran J Basic Med Sci 20(4):338

Bal A, Panda F, Pati SG, Das K, Agrawal PK, Paital B (2021) Modulation of physiological oxidative stress and antioxidant status by abiotic factors especially salinity in aquatic organisms. Comp Biochem Physiol Part C Toxical Pharmacol 241:108971

Birnie-Gauvin K, Costantini D, Cooke SJ, Willmore WG (2017) A comparative and evolutionary approach to oxidative stress in fish: a review. Fish Fish 18(5):928–942

Bolner K, Baldisserotto B (2007) Water pH and urinary excretion in silver catfish Rhamdia quelen. J Fish Biol 70(1):50–64

Chen X, Liu S, Ding Q, Teame T, Yang Y, Ran C, Zhang Z, Zhou Z (2023) Research advances in the structure, function, and regulation of the gill barrier in teleost fish. Water Biology Security:100139

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. Aquaculture Fisheries 7(4):389–395
Churcher AM, Pujolar JM, Milan M, Hubbard PC, Martins RS, Saraiva JL, Huertas M, Bargelloni L, Patarnello T, Marino IA (2014) Changes in the gene expression profiles of the brains of male European eels (Anguilla anguilla) during sexual maturation. BMC Genomics 15:1–13
Ciji A, Akhtar MS (2021) Stress management in aquaculture: a review of dietary interventions. Rev Aquac 13(4):2190–2247
Copatti CE, Baldisserotto B, Souza CdF, Monserrat JM, Garcia L (2019) Water pH and hardness alter ATPases and oxidative stress in the gills and kidney of pacu (Piaractus mesopotamicus). Neotropical Ichthyol 17(4):e190032
da PaixaoLemos CH, Ribeiro CVDM, de Oliveira CPB, Couto RD, Copatti CE (2018) Effects of interaction between pH and stocking density on the growth, haematological and biochemical responses of Nile tilapia juveniles. Aquaculture 495:62–67
Ding L, Liu Y, Wei X, Geng C, Liu W, Han L, Yuan F, Wang P, Sun Y (2023) Effects of saline-alkaline stress on metabolome, biochemical parameters, and histopathology in the kidney of crucian carp (Carassius auratus). Metabolites 13(2):159
Dou Z, Liu C, Feng X, Xie Y, Yue H, Dong J, Zhao Z, Chen G, Yang J (2022) Camel whey protein (CWP) ameliorates liver injury in type 2 diabetes mellitus rats and insulin resistance (IR) in HepG2 cells via activation of the PI3K/Akt signaling pathway. Food Function 13(1):255–269
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(26):10248–10253
Fossati P, Prencipe L, Berti G (1983) Enzymic creatinine assay: a new colorimetric method based on hydrogen peroxide measurement. Clin Chem 29(8):1494–1496. https://doi.org/10.1093/clinchem/29.8.1494/JClinicalChemistry
Gan L, Xu Z, Ma J, Xu C, Wang X, Chen K, Chen L, Li E (2016) Effects of salinity on growth, body composition, muscle fatty acid composition, and antioxidant status of juvenile Nile tilapia Oreochromis niloticus (Linnaeus, 1758). J Appl Ichthyol 32 (2)
Ge Q, Wang J, Li J, Li J (2023) Effect of high alkalinity on shrimp gills: histopathological alternations and cell specific responses. Ecotoxicol Environ Saf 256:114902
Geletu TT, Zhao J (2023) Genetic resources of Nile tilapia (Oreochromis niloticus Linnaeus, 1758) in its native range and aquaculture. Hydrobiologia 850(10):2425–2445
Geng L, Tong G, Jiang H (2016) Xu W (2016) Effect of salinity and alkalinity on luciobarbus capito Gill Na+/K+-ATPase enzyme activity, plasma ion concentration, and osmotic pressure. BioMed Res Int 1:4605839
Hamed H, Bellassoued k, El Feki A, Gargouri A, (2019) Evaluation of the hepatoprotective effect of combination between fermented camel milk and Rosmarinus officinalis leaves extract against CCl 4 induced liver toxicity in mice. J Food SciTechnol 56:824–834
Hoyle NT, Merritt JH (1994) Quality of fish protein hydrolysates from herring (Clupea harengus). J Food Sci 59:76–79
Huang S, Li C, Li Z, Duanzhi D, Liu Y, Ran F, Ding J, Yan W, Jia C, Zhang Z (2022) Short-term exposure to 5‰ and 15‰ salinity causes the dynamic changes of the NKA gene, enzyme activities and morphological characteristics in fish tissues of Gymnocypris przewalskii. Aquac Res 53(17):6389–6398
Ibrahim HR, Isono H, Miyata T (2018) Potential antioxidant bioactive peptides from camel milk proteins. Animal Nutrition 4(3):273–280
Ibrahim RE, Elshopakey GE, Abd El-Rahman GI, Ahmed AI, Altohamy DE, Zaglool AW, Younis EM, Abdelwarith AA, Davies SJ, Al-Harthi HF (2022) Palliative role of colloidal silver nanoparticles synthetized by moringa against Saprolegnia spp. infection in Nile tilapia: biochemical, immunoantioxidant response, gene expression, and histopathological investigation. Aquaculture Reports 26:101318
Ibrahim RE, Elshopakey GE, Aly MY, Abdelwarith AA, Younis EM, Abd-Elhakim YM, Khamis T, Osman A, Metwally MM, Davies SJ (2024) 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. Aquac Int:1–22
Izadi A, Khedmat L, Mojtahedi SY (2019) Nutritional and therapeutic perspectives of camel milk and its protein hydrolysates: a review on versatile biofunctional properties. J Funct Foods 60:103441
Jafar S, Kamal H, Mudgil P, Hassan HM, Maqsood S (2018) Camel whey protein hydrolysates displayed enhanced cholesteryl esterase and lipase inhibitory, anti-hypertensive and anti-haemolytic properties. LWT 98:212–218
Jiang Y, Yuan C, Qi M, Liu Q, Hu Z (2022) The effect of salinity stress on enzyme activities, histology, and transcriptome of silver carp (Hypophthalmichthys molitrix). Biology 11(11):1580
Katikaneni A, Jelcic M, Gerlach GF, Ma Y, Overholtzer M, Niethammer P (2020) Lipid peroxidation regulates long-range wound detection through 5-lipoxygenase in zebrafish. Nat Cell Biol 22(9):1049–1055
Kaushal SS, Likens GE, Pace ML, Utz RM, Haq S, Gorman J, Grese M (2018) Freshwater salinization syndrome on a continental scale. Proc Natl Acad Sci 115(4):E574–E583
Kim B-M, Rhee J-S, Jeong C-B, Seo JS, Park GS, Lee Y-M, Lee J-S (2014) Heavy metals induce oxidative stress and trigger oxidative stress-mediated heat shock protein (hsp) modulation in the intertidal copepod Tigriopus japonicus. Comp Biochem Physiol Part C Toxicol Pharmacol 166:65–74
Kolesarova A, Slamecka J, Jurcik R, Tataruch F, Lukac N, Kovacik J, Capcarova M, Valent M, Massanyi P (2008) Environmental levels of cadmium, lead and mercury in brown hares and their relation to blood metabolic parameters. Journal of Environmental Science Health, Part A 43(6):646–650
Kovacik A, Arvay J, Tusimova E, Harangozo L, Tvrda E, Zbynovska K, Cupka P, Andrascikova S, Tomas J,

Massanyi P (2017) Seasonal variations in the blood concentration of selected heavy metals in sheep and their effects on the biochemical and hematological parameters. Chemosphere 168:365–371

Kumar D, Chatli MK, Singh R, Mehta N, Kumar P (2016) Enzymatic hydrolysis of camel milk casein and its antioxidant properties. Dairy Sci Technol 96:391–404

Li M, Kong Y, Wu X, Yin Z, Niu X, Wang G (2021) Dietary α-lipoic acid can alleviate the bioaccumulation, oxidative stress, cell apoptosis, and inflammation induced by lead (Pb) in Channa argus. Fish Shellfish Immunol 119:249–261

Liu Y, Yao M, Li S, Wei X, Ding L, Han S, Wang P, Lv B, Chen Z, Sun Y (2022) Integrated application of multi-omics approach and biochemical assays provides insights into physiological responses to saline-alkaline stress in the gills of crucian carp (*Carassiusauratus*). Sci Total Environ 822:153622

Lushchak VI (2011) Environmentally induced oxidative stress in aquatic animals. Aquat Toxicol 101(1):13–30

Magbanua TO, Ragaza JA (2024) Selected dietary plant-based proteins for growth and health response of Nile tilapia Oreochromis niloticus. Aquaculture Fisheries 9(1):3–19

Marx M, Souza C, Almeida A, Descovi S, Bianchini A, Martos-Sitcha J, Martínez-Rodríguez G, Antoniazzi A, Baldisserotto B (2022) Expression of ion transporters and Na+/K+-ATPase and H+-ATPase activities in the gills and kidney of silver catfish (Rhamdia quelen) exposed to different pHs. Fishes 2022, 7, 261. s Note: MDPI stays neutral with regard to jurisdictional claims in published …,

Massanyi P, Stawarz R, Halo M, Formicki G, Lukac N, Cupka P, Schwarcz P, Kovacik A, Tusimova E, Kovacik J (2014) Blood concentration of copper, cadmium, zinc and lead in horses and its relation to hematological and biochemical parameters. Journal of Environmental Science Health, Part A 49(8):973–979

Neiffer DL, Stamper MAJIj (2009) Fish sedation, anesthesia, analgesia, and euthanasia: considerations, methods, and types of drugs. 50 (4):343–360

NRC (2011) Nutrient requirements of fish and shrimp. National academies press.

Nwizugbo KC, Ogwu MC, Eriyamremu GE, Ahana CM (2023) Alterations in energy metabolism, total protein, uric and nucleic acids in African sharptooth catfish (Clarias gariepinus Burchell) exposed to crude oil and fractions. Chemosphere 316:137778

Okasha LA, Abdellatif JI, Abd-Elmegeed OH, Sherif AH (2024) Overview on the role of dietary Spirulina platensis on immune responses against Edwardsiellosis among Oreochromis Niloticus fish farms. BMC Vet Res 20:290

Osman A, El-Hadary A, Korish AA, AlNafea HM, Alhakbany MA, Awad AA, Abdel-Hamid M (2021) Angiotensin-I converting enzyme inhibition and antioxidant activity of papain-hydrolyzed camel whey protein and its hepatorenal protective effects in thioacetamide-induced toxicity. Foods 10(2):468

Romero MF (2005) Molecular pathophysiology of SLC4 bicarbonate transporters. Curr Opin Nephrol Hypertens 14(5):495–501

Salami M, Moosavi-Movahedi AA, Moosavi-Movahedi F, Ehsani MR, Yousefi R, Farhadi M, Niasari-Naslaji A, Saboury AA, Chobert J-M, Haertlé T (2011) Biological activity of camel milk casein following enzymatic digestion. J Dairy Res 78(4):471–478

Saliu J, Oluberu S, Akpoke I, Ukwa UJAJoAS (2017) Cortisol stress response and histopathological alteration index in Clarias gariepinus exposed to sublethal concentrations of Qua Iboe crude oil and rig wash. 42 (1):55–64

Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3(6):1101–1108

Sherif AH, Elshenawy AM, Attia AA, Salama SS (2021) Effect of Aflatoxin B1 on farmed *Cyprinuscarpio* in conjunction with bacterial infection. Egyptian J Aquat Biol Fish 25:465–485

Sherif AH, Farag EAH, Mahmoud AE (2024) Temperature fluctuation alters immuno-antioxidant response and enhances the susceptibility of *Oreochromisniloticus* to *Aeromonashydrophila* challenge. Aquac Int 32:2171–2184

Shin H-S, An K-W, Kim N-N, Choi C-Y (2010) Antioxidant defenses and physiological changes in olive flounder (Paralichthys olivaceus) in response to oxidative stress induced by elevated water temperature. Korean J Ichthyol 22(1):1–8

Suvarna KS, Layton C, Bancroft JD (2018) Bancroft's theory and practice of histological techniques. Elsevier health sciences,

Takvam M, Wood CM, Kryvi H, Nilsen TO (2021) Ion transporters and osmoregulation in the kidney of teleost fishes as a function of salinity. Front Physiol 12:664588

Tang C, Lee T (2007) The effect of environmental salinity on the protein expression of Na+/K+-ATPase, Na+/K+/2Cl− cotransporter, cystic fibrosis transmembrane conductance regulator, anion exchanger 1, and chloride channel 3 in gills of a euryhaline teleost, Tetraodon nigroviridis. Comp Biochem Physiol Part a: Mol Int Physiol 147(2):521–528

Trinder P (1969) Determination of blood glucose using 4-amino phenazone as oxygen acceptor. J Clin Pathol 22(2):246

Wen B, Jin S-R, Chen Z-Z, Gao J-Z (2018) Physiological responses to cold stress in the gills of discus fish (Symphysodon aequifasciatus) revealed by conventional biochemical assays and GC-TOF-MS metabolomics. Sci Total Environ 640:1372–1381

Wilkie MP, Wood CM (1996) The adaptations of fish to extremely alkaline environments. Comp Biochem Physiol Part b: Biochem Mol Biol 113(4):665–673

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(3):397–402

Zhang Y, Wen H, Liu Y, Qi X, Sun D, Zhang C, Zhang K, Zhang M, Li J, Li Y (2023) 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. Comparative Biochemistry Physiology 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