Aquaculture Reports 22 (2022) 100984

Contents lists available at ScienceDirect

Aquaculture Reports

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

Dietary *Bacillus subtilis* relieved the growth retardation, hepatic failure, and antioxidative depression induced by ochratoxin A in Thinlip Mullet (*Liza ramada*)

Fawzy Magouz [a], Hasnaa Abu-Ghanima [a], Amr I. Zaineldin [b], Mahmoud S. Gewaily [c], Ali Soliman [d], Asem A. Amer [e], Eman M. Moustafa [f], Elsayed M. Younis [g], Abdel-Wahab A. Abdel-Warith [g], Simon J. Davies [h], Hien Van Doan [i,j,*], Wanaporn Tapingkae [i], Mahmoud A. O. Dawood [a,k,**]

[a] Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt
[b] Animal Health Research Institute (AHRI-DOKI), Agriculture Research Center, Egypt
[c] Department of Anatomy and Embryology, Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt
[d] Fish Nutrition Laboratory, Aquaculture Division, National Institute of Oceanography and Fisheries, Alexandria, Egypt
[e] Central Laboratory for Aquaculture Research, Abbassa, Sharkia, Sakha Aquaculture Research Unit, Kafrelsheikh, Egypt
[f] Department of Fish Diseases and Management, Faculty of Veterinary Medicine Kafrelsheikh University, Egypt
[g] Department of Zoology, College of Science, King Saud University, PO Box 2455, Riyadh 11451, Kingdom of Saudi Arabia
[h] School of Science and Engineering, National University of Ireland Galway, Republic of Ireland
[i] Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[j] Innovative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
[k] The Center for Applied Research on the Environment and Sustainability, The American University in Cairo, 11835 Cairo, Egypt

ARTICLE INFO

Keywords:
Aquafeed
Feed additives
Mycotoxicosis
Lipid peroxidation
Histology

ABSTRACT

Mycotoxicosis is a severe challenge in the aquafeed industry and is involved in low productivity and high economic loss. On this occasion, available dietary supplements, including probiotics, may relieve the impacts of mycotoxicosis on the performances of aquatic animals. In this study, four test diets were prepared to test the effects of *Bacillus subtilis* (BS), ochratoxin A (OTA), and their mixture (BS/OTA) on the performances of Mullet (*Liza ramada*). Fish of similar initial weight (11.38 ± 0.24 g) were divided into four groups stocked in triplicate hapas (0.5 ×0.5 ×1 m) at 15 fish per hapa. Fish fed the control diet (without BS or OTA), BS (2×10^6 CFU/g), OTA (1 mg/kg) or BS (2×10^6 CFU/g) and OTA (1 mg/kg) (BS/OTA). After eight weeks, the final weight (FBW) and specific growth rate (SGR) were markedly enhanced by dietary BS and reduced by OTA contamination, while the feed conversion ratio (FCR) was meaningfully reduced by dietary BS and increased by OTA. The hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBCs), and white blood cells (WBCs) were markedly lowered by OTA toxicity. Fish fed BS, or BS/OTA diets showed no significant differences with the control in terms of Hb, PCV, RBCs, and WBCs ($p > 0.05$). The BS-fed fish showed the same normal intestinal and liver structure with an improved appearance of intestinal villi. The OTA-exposed fish showed deterioration of intestinal mucosa and stunted growth of intestinal villi with severe liver vascular dilatation and congestion in addition to hepatocytes degeneration. The combination of BS with OTA alleviated the pathological effect of individual OTA on the intestinal villi, improved intestinal morphology, and restored the normal hepatic structures with mild periductal inflammatory reaction around the bile duct. The blood total protein and albumin levels were markedly increased by dietary BS and lowered by OTA. Fish fed BS/OTA diet had higher alanine aminotransferase (ALT), aspartate aminotransferase (AST), and urea than fish fed the control but lower than those contaminated with OTA. The catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were markedly increased by dietary BS and lowered by OTA toxicity. However, the malondialdehyde (MDA) level was decreased by dietary BS and increased by OTA toxicity. Interestingly, fish fed the control and BS/OTA diet had similar FBW, SGR, FCR, survival rates, Hb, PCV, RBCs, WBCs, creatinine, CAT, GPx, SOD, and MDA levels. In conclusion, dietary

* Corresponding author at: Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand.
** Corresponding author at: Department of Animal Production, Faculty of Agriculture, Kafrelsheikh University, Kafrelsheikh, Egypt.
E-mail addresses: hien.d@cmu.ac.th (H. Van Doan), Mahmoud.dawood@agr.kfs.edu.eg (M.A.O. Dawood).

https://doi.org/10.1016/j.aqrep.2021.100984
Received 17 October 2021; Received in revised form 9 December 2021; Accepted 17 December 2021
Available online 21 December 2021

B. subtilis relieved the negative impacts of OTA contamination via protecting fish's intestine, liver, and kidney function.

1. Introduction

Sustainable aquaculture practices ensure the necessity of securing high-quality feeds (Kari et al., 2021). However, the cost of aquafeed is increased marginally with the high demand and low availability of fishmeal (Dawood, 2021). Alternatively, plant-based feeds are increasingly formulated to fulfill the requirements of aquatic animals (Glencross et al., 2020). Nevertheless, this substitution increases the threat of mycotoxin contamination, which is a critical challenge for both fish and human health (Pietsch, 2020; Zahran et al., 2020). Particularly ochratoxin-A (OTA), a very toxic plant protein-borne mycotoxin produced by many Penicillium and *Aspergillus* species (Gonçalves et al., 2020). Several filamentous fungi can grow on the harvested grains, cereals, and seeds and result in high levels of OTA secretion (Akinmusire et al., 2019). OTA contamination caused severe negative impacts on aquatic animals' production and health conditions (Meucci et al., 2021). OTA combines with serum albumin with direct binding, resulting in an extended serum half-life of 35 days (Bui-Klimke and Wu, 2015). Markedly, OTA contamination resulted in retarded growth performance, neurotoxicity, cytotoxicity, impaired immunity, and disrupted physiological functions (Bernhoft et al., 2017; Wu et al., 2020). Besides, injuries in the gills, intestines, kidneys, and liver induced by OTA were seen in aquatic animals (Bernhoft et al., 2017; Liu et al., 2021). Thus, high mortality rates and substantial economic loss resulted from the contamination of aquafeed with OTA (Baldissera et al., 2020a; Tschirren et al., 2018). Adsorbing or binding compounds have frequently been employed to decrease OTA contamination by binding with mycotoxins; however, adsorbents may reduce feed efficiency and mineral utilization (Fadl et al., 2020). Noteworthy that adsorbents can bind to minerals and vitamins in the basal diet leading to low availability (20% shortage) (Brown et al., 2014). Hence, using functional additives is recently suggested to enhance the adsorption of mycotoxins and relieve their negative impacts on the health status of aquatic animals.

Incorporating beneficial microorganisms to relieve the impacts of mycotoxins on aquatic animals is another appropriate strategy (El-Saadony et al., 2021). Biological anti-mycotoxins are effective, bioavailable, safe, and feasible additives in aquaculture (Pinheiro et al., 2020). *Bacillus subtilis* is a Gram-positive bacteria characterized by rod-shaped, endospore-forming aerobic or facultatively anaerobic bacteria (Logan and Halket, 2011). *B. subtilis* is widely utilized as an efficient probiotic supplement, resulting in enhanced growth, intestinal microbiota diversity, physiological function, antioxidative, and immune responses in aquaculture (Cao et al., 2019; Wang et al., 2021). A plethora of studies investigated the potential roles of *B. subtilis* for enhancing productivity, health status, and resistance against infection with invaders (Di et al., 2019; Li et al., 2021; Liu et al., 2017; Tang et al., 2019; Xue et al., 2020). Recently, Fan et al. (2018) reported that dietary *B. subtilis* could relieve the impacts of aflatoxicosis on Yellow River carp performances. The results displayed protected intestinal and liver tissues with regulated digestive enzyme activity resulting from *B. subtilis* supplementation.

Thinlip Mullet (*Liza ramada*) is a valuable candidate for aquaculture in Europe and the Mediterranean (Shukry et al., 2021). However, the farming of L. *ramada* is threatened with mycotoxin contamination. Herein, the study evaluated the effects of feed-borne OTA on the performances, hepato-renal function, intestine and liver histological features, and antioxidative capacity of L. *ramada*. Further, the study investigated the possible protective roles of *B. subtilis* supplementation.

2. Materials and methods

2.1. Ethical statement

The study was conducted according to the Ethics Committee of Faculty of Agriculture guidelines, Kafrelsheikh University, Egypt (No. 07/12/2020EC).

2.2. Experimental fish

The juveniles of Thinlip Mullet (*Liza ramada*) were collected from Bughaz El-Burullus (Lake Burullus, Baltim city, Kafr El-sheikh, Egypt) and carefully transported to the Fish Nutrition Laboratory, Baltim Unit, National Institute of Oceanography and Fisheries. Fish were kept in a concrete tank (3 ×2 ×1.7 m) and fed the basal control diet twice daily (08:00 and 15:00). Underground water was stocked in concrete tanks until used with 12 ppt salinity. The water was running in a flow-through system in the outdoor area. After two weeks, fish of similar initial weight (11.38 ± 0.24 g) were divided into 12 hapas (0.5 ×0.5 ×1 m) at 15 fish per hapa. All hapas were fixed with continuous water inlets and outlets in one concrete tank. For eight weeks, fish were fed the diets at 2–3% twice daily (08:00 and 15:00). The water characteristics were recorded: temperature (27.11 ± 0.35 °C), pH (7.22 ± 0.27), dissolved oxygen (5.88 ± 0.32 mg/L), salinity (12 ppt), and total ammonia (0.15 ± 0.02 mg/L).

2.3. Diet preparation

The basal diet (control) is made from fish meal, soybean meal, yellow corn, gluten, wheat bran, rice bran, wheat flour, fish oil, and vitamin and mineral mixture (Table 1). The ingredients were well mixed, then water was added. In the case of the second diet, lyophilized *Bacillus subtilis* (BS) was supplemented in the basal diet at 2×10^6 CFU/g (2×10^9 CFU/g, Free Trade Egypt Company, Elbehiera, Egypt). The dose of BS was adjusted by using wheat flour, as described earlier by Du et al. (2021). Bacterial cells were stocked using tryptic soy agar (TSA) and incubated at 35 °C for 24 h. Bacterial cells were then centrifuged at 1008 ×*g* for 30 min to form pellets, re-suspended in 1.0 ml of sterile water 2×10^6 CFU/g (Du et al., 2021). The ingredients of the basal diet were mixed thoroughly with distilled water and suspended bacterial strain. After completely air dry, pellets were collected and kept at − 20 °C until used. The third diet was mixed with Ochratoxin-A (OTA, Sigma-Aldrich™, St. Louis, Missouri, USA) at 1 mg/kg (Manning et al., 2003). The fourth diet supplemented BS (2×10^6 CFU/g) and OTA (1 mg/kg). Subsequently, all ingredients were pelleted in a pelletizer machine with a 2 mm die, kept drying at room temperature, and then kept in plastic bags at 4 °C until further use. The presence and dosage of BS in the test diets were confirmed by following Addo et al. (2017) using tryptic soy agar (TSA). The typical morphological characteristics (e.g., rod-shaped, endospore-forming aerobic or facultatively anaerobic, Gram-positive bacteria) were considered indicators for BS viability. Viable spore counts are done by serial decimal dilutions in distilled sterile water and inoculated on TSA surface in duplicate. Plates are incubated overnight at 36 °C. Formulated diets were thoroughly homogenized in a mortem, and OTA was extracted, filtrated, and analyzed quantitatively by HPLC (Fadl et al., 2020; Hathout et al., 2020). In brief, samples (50 g) were well-grounded and blended with acetonitrile (Merck, Darmstadt, Germany): water (60:40) (100 ml) at high speed for 1 min. Then, the solution was filtered with fluted filter paper (Whatman No. 4, Whatman, Cytiva, Marlborough, MA 01752, USA). The purified extract (10 ml) was diluted with Phosphate Buffer Saline (PBS) (40 ml) and mixed well, then

Table 1
Basal diet and proximate chemical composition (%, on dry matter basis).

Ingredients[a]	Control	BS	OTA	BS/OTA
Fish meal	8	8	8	8
Soybean meal (44% cp)	35	35	35	35
Yellow corn	15	15	15	15
Gluten	15	15	15	15
Wheat bran	10	10	10	10
Rice bran	7	7	7	7
Wheat flour	4.9	4.9	3.9	3.9
Fish oil	2	2	2	2
Vitamin and mineral mixture[b]	1	1	1	1
Dicalcium phosphate	2	2	2	2
Vitamin C	0.1	0.1	0.1	0.1
Ochratoxin-A (OTA) (mg/kg)	0	0	1	1
Bacillus subtilis (BS, CFU/g)	0	1×10^6	0	1×10^6
Total (%)	100	100	100	100
Content				
Crude protein (%)	30.15	30.24	30.37	30.19
Crude lipids (%)	5.92	5.83	5.78	5.84
Ash (%)	6.34	6.42	6.51	6.67
Fibers (%)	6.11	6.24	6.09	6.19
Gross energy (MJ/kg)[c]	18.31	18.26	18.27	18.22
Ochratoxin-A (OTA) (mg/kg)	0	0	1	1
Bacillus subtilis (CFU/g)	0.00	1×10^6	0.00	1×10^6

[a] Supplied by Feed Control Co., Ltd. (Damro, Sidi Salem, Kafrelsheikh, Egypt). Dicalcium phosphate, vitamin C, methionine, L-lysine, threonine, tryptophan (DSM in Animal Nutrition & Health, Heerlen, the Netherlands).

[b] Vitamin mixture (mg/kg premix): vitamin A (3300 IU), vitamin D_3 (410 IU), vitamin E (2660 mg), vitamin B_1 (133 mg), vitamin B_2 (580 mg), vitamin B_6 (410 mg), vitamin B_{12} (50 mg), biotin (9330 mg), colin chloride (4000 mg), inositol (330 mg), para-amino benzoic acid (9330 mg), niacin (26.60 mg), pantothenic acid (2000 mg); mineral mixture (mg/kg premix): manganese (325 mg), iron (200 mg), copper (25 mg), iodine, cobalt (5 mg).

[c] Gross energy was calculated based on protein, lipid, and carbohydrate values as 23.6, 39.5, and 17.2 KJ/g, respectively.

filtered through a glass microfiber filter. Afterward, 10 ml of the extract was passed entirely through the immunoaffinity column (OchraTes, Vicam, Milford, MA 01757, USA) (1 drop/second). The eluate was eluted using HPLC grade methanol (1.5 ml), then purified water (1.5 ml) was added, vortexed, and analyzed by HPLC. OTA standards were purchased from Sigma-Aldrich™ (Saint Louis, MO 63103, USA). The chemical composition of the prepared diets was checked by following AOAC (2012).

2.4. Final sampling

After eight weeks, fish were starved 24 h, then fish were individually weighed (FBW, g) and counted to calculate the growth performance and survival rate.

$$\text{WG (\%)} = 100 \times (\text{FBW} - \text{initial weight (IBW, g)})/\text{IW (g)}$$

$$\text{SGR (\% IBW / day)} = 100 \times (\ln \text{FBW (g)} - \ln \text{IBW (g)})/\text{days}$$

$$\text{FCR} = \text{total dry feed intake (FI, g)}/ (\text{FBW (g)} - \text{IBW (g)})$$

$$\text{Survival (\%)} = 100 \times \text{final fish number/initial fish number}$$

Where, WG: weight gain; SGR: specific growth rate; FCR: feed conversion ratio; IBW: initial body weight; FBW: final body weight.

Then fish were anesthetized with 100 mg/L tricaine methanesulfonate, and blood was collected from 3 fish/hapa using 5 ml gauge syringes from the caudal vein. Half of the blood was kept in EDTA-heparinized tubes and immediately used for hematological analysis. The remaining blood was kept in non-heparinized tubes for serum collection. After 2 h, blood samples were centrifuged at 1008 × *g*/ 15 min at 4 °C (SCILOGEX, Model: DM0412, USA); then, serum was separated and kept at − 20 °C for further analysis. Besides, three fish per hapa were dissected, and their intestines and livers were dissected to analyze the histological features.

2.5. Blood analysis

The total white blood cell (WBC) and red blood cell (RBC) counts were determined by a haemocytometer (Houston, 1990). Hemoglobin (Hb) values were determined according to Collier (1944), while the packed cell volume (PCV, %) values using the microhematocrit method. The mean corpuscular hemoglobin concentration (MCHC) was estimated as described by Wintrobe (1934). The mean corpuscular volume (MCV) was directly measured by an automated Coulter LH 750 hematology analyzer (Beckman Coulter, Fullerton, CA) (Dacie and Lewis, 1995). The differential of white blood cell (WBC) counts done according to Klontz (1994).

Serum total proteins and albumins were determined, according to Doumas et al. (1981) and Dumas and Biggs (1972). Globulin is calculated from the difference between total protein and albumin values. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), creatinine, urea, and uric acid were detected by RA-50 chemistry analyzer (Bayer) using readymade chemicals (kits) supplied by Spinreact Co. Spain, following the manufacturer's instructions.

Superoxide dismutase (McCord and Fridovich, 1969), catalase, and glutathione peroxidase (Habig et al., 1974) in serum samples were measured using diagnostic reagent kits following the manufacturer's (Biodiagnostic Co., Giza, Egypt) instructions. The malondialdehyde (MDA) concentration was detected in serum by following Uchiyama and Mihara (1978) and expressed as nmol MDA/ml. Briefly, samples (10%, w/v) were mixed with 1.5 ml of 1% H_3PO_4 and 0.5 ml of 0.6% thiobarbituric acid. The tubes were heated for 60 min in a boiling water bath. After cooling in an ice bath, 2 ml of butanol were added, and contents were mixed vigorously for 20 s. After centrifugation (1107 g, 15 min), the absorbance of the organic layer was measured at 520 and 535 nm using a spectrometer (Lambda 2 S, Perkin-Elmer Co., USA).

2.6. Histomorphology

The histopathological samples (intestine and liver) were fixed in 10% neutral buffered formalin. After 24 h, the samples were extensively transferred to 70% alcohol. The tissue sections were prepared following the conventional staining of hematoxylin and eosin (H&E) as previously described (Gewaily and Abumandour, 2021). The stained sections were examined with a BX50/BXFLA microscope (Olympus, Tokyo, Japan). The stained sections were examined under a light microscope (Leica DM500; Leica Microsystems, Japan).

2.7. Statistical analysis

Shapiro-Wilk and Levene tests confirmed normal distribution and homogeneity of variance. The obtained data were subjected to one-way ANOVA. Differences between means were tested at $p < 0.05$ level using the Duncan test as a post-doc test. Two-way ANOVA analysis was performed to detect the effects of BS, OTA, and their mixture (BS/OTA) on the performances of L. *ramada*. All the statistical analyses were done via SPSS version 22 (SPSS Inc., IL, USA).

3. Results

3.1. Growth performance

The final weight (FBW) and specific growth rate (SGR) were markedly enhanced by dietary BS and reduced by OTA contamination (Table 2). On the other hand, the feed conversion ratio (FCR) was meaningfully reduced by dietary BS while increased by OTA. The survival rate showed the highest being in the group of fish fed BS (97.78 ± 2.22%) and the lowest being in the group contaminated with OTA (84.45 ± 2.22%) (Table 2). Interestingly, fish fed BS and contaminated

Table 2
Growth performance of *Liza ramada* fed diets contaminated with OTA or/and *Bacillus subtilis* (BS).

Item	Groups				Two-way ANOVA (*p* value)		
	Control	BS	OTA	BS/OTA	BS	OTA	BS×OTA
IBW (g)	11.29 ± 0.05	11.24 ± 0.11	11.67 ± 0.08	11.69 ± 0.09	NS	NS	NS
FBW (g)	33.18 ± 0.55^{b}	38.55 ± 0.74^{a}	27.52 ± 0.39^{c}	32.77 ± 0.26^{b}	0.001	0.001	0.001
WG (%)	193.96 ± 4.08^{b}	243.00 ± 5.88^{a}	135.95 ± 4.14^{d}	180.38 ± 0.58^{c}	0.001	0.001	0.001
SGR (%/day)	1.80 ± 0.02^{b}	2.06 ± 0.03^{a}	1.43 ± 0.03^{c}	1.72 ± 0.00^{b}	0.001	0.001	0.001
FI (g/fish)	27.78 ± 0.83	30.49 ± 0.71	30.11 ± 1.10	27.55 ± 1.01	NS	NS	NS
FCR	1.27 ± 0.05^{b}	1.12 ± 0.04^{c}	1.89 ± 0.13^{a}	1.31 ± 0.01^{b}	0.001	0.001	0.001
Survival (%)	95.55 ± 2.22^{b}	97.78 ± 2.22^{a}	84.45 ± 2.22^{c}	93.33 ± 0.00^{b}	0.001	0.001	0.001

Values are presented as means ± S.E. (n = 3). Different superscript letters refer to significant differences ($p < 0.05$). OTA: Ochratoxin-A; IBW: initial weight (g); FBW: final weight (g); WG: weight gain (%); SGR: specific growth rate (%/day); FI: feed intake (g/fish); FCR: feed conversion ratio; NS: not significant ($p > 0.05$).

with OTA had similar FBW, SGR, FCR, and survival rates to the control group. The two-way ANOVA illustrated that the BS, OTA, and BS/OTA were synergistically significant factors ($p < 0.05$) on the FBW, WG, SGR, FCR, and survival rate (Table 2).

3.2. Hematological profile

The hematological profile of L. *ramada* fed BS and contaminated with OTA is presented in Table 3. The Hb, PCV, RBCs, and WBCs were markedly increased by dietary BS and lowered by OTA toxicity. Fish fed BS and contaminated with OTA had similar Hb, PCV, RBCs, and WBCs to the control. No marked effects for BS and/or OTA were seen on the remaining hematological profile. The two-way ANOVA illustrated that the BS, OTA, and BS/OTA were synergistically significant factors ($p < 0.05$) on the Hb, PCV, RBCs, and WBCs (Table 3).

3.3. Histological study

The histopathological investigation of the intestine in the control group revealed normal intact intestinal mucosa with intestinal villi and other layers of intestinal wall; submucosa, muscularis, and serosa (Fig. 1; A). The BS-fed fish showed the same normal structure with improved appearance of intestinal villi where the villus height, width, and villus area were increased and enhanced thickness of mucosa and muscularis (Fig. 1; B). OTA-exposed fish showed deterioration of intestinal mucosa and stunted growth of intestinal villi (Fig. 2; C). The combination of BS with OTA alleviated the pathological effect of individual OTA on the intestinal villi and improved intestinal morphology (Fig. 1; D).

Histopathological appearance of the liver in control and BS-fed fish demonstrated normal hepatic cells with vesicular, centrally located nuclei, increased glycogen deposit inside the hepatocytes. The hepatic blood sinusoids were not dilated or congested between hepatocytes which arranged in the cord-like appearance (Fig. 2; A, B). The OTA-subjected fish showed severe vascular dilatation and congestion in addition to hepatocytes vacuolation, degeneration, and nuclear pyknosis (Fig. 2; C). The addition of BS with OTA prevented the degenerative changes and restored the normal hepatic structures with mild periductal inflammatory reaction around the bile duct (Fig. 2; D).

3.4. Blood biochemical profile

The blood total protein and albumin levels were markedly increased by dietary BS and lowered by OTA (Fig. 3). The globulin level was higher in fish of control and BS groups than in the OTA group without differences with the BS/OTA group (Fig. 3). Fish contaminated with OTA had the highest ALT and AST, while fish-fed dietary BS had the lowest ALT and AST (Fig. 3). Fish fed the control and BS diets had the lowest urea and creatinine levels, but those contaminated with OTA had the highest urea and creatinine levels (Fig. 3). Fish fed BS/OTA diet had higher ALT, AST, and urea than fish fed the control but lower than those contaminated with OTA. Interestingly, fish fed the control and BS/OTA diet had similar creatinine levels. The two-way ANOVA illustrated that the BS, OTA, and BS/OTA were synergistically significant factors ($p < 0.05$) on all detected blood biochemical traits (Fig. 3).

3.5. Antioxidative capacity

The antioxidative capacity of L. *ramada* fed BS and contaminated with OTA is presented in Fig. 4. The catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) were markedly increased by dietary BS and lowered by OTA toxicity. However, the malondialdehyde (MDA) level was decreased by dietary BS and increased by OTA toxicity. Fish fed BS and contaminated with OTA had

Table 3
Hematological profile of *Liza ramada* fed diets contaminated with OTA or/and *Bacillus subtilis* (BS).

Item	Groups				Two-way ANOVA (*p* value)		
	Control	BS	OTA	BS/OTA	BS	OTA	BS×OTA
Hb (g/100 ml)	12.12 ± 0.06^{ab}	12.32 ± 0.11^{a}	10.64 ± 0.14^{c}	11.48 ± 0.06^{b}	0.001	0.001	0.001
RBCs (×10^{6}/mm^{3})	3.69 ± 0.05^{ab}	4.05 ± 0.06^{a}	3.09 ± 0.08^{c}	3.53 ± 0.03^{b}	0.001	0.001	0.001
PCV (%)	37.40 ± 0.19^{ab}	39.14 ± 0.33^{a}	34.31 ± 0.30^{c}	35.84 ± 0.33^{b}	0.001	0.001	0.001
MCV (μm^{3}/cell)	101.53 ± 1.41	96.63 ± 1.43	111.24 ± 2.81	101.67 ± 1.46	NS	NS	NS
MCH (pg/cell)	32.90 ± 0.53	30.41 ± 0.51	34.47 ± 0.71	32.57 ± 0.25	NS	NS	NS
MCHC (%)	32.40 ± 0.27	31.48 ± 0.46	31.02 ± 0.47	32.04 ± 0.28	NS	NS	NS
WBCs (×10 ^{3}/mm^{3})	36.68 ± 0.50^{ab}	39.29 ± 0.27^{a}	32.53 ± 0.42^{c}	34.78 ± 0.47^{b}	0.001	0.001	0.001
Heterophil (%)	6.60 ± 0.25	7.20 ± 0.20	5.80 ± 0.37	6.40 ± 0.25	NS	NS	NS
Lymphocyte (%)	81.60 ± 0.60	83.00 ± 0.32	78.20 ± 0.37	79.80 ± 0.20	NS	NS	NS
Monocyte (%)	5.80 ± 0.20	4.60 ± 0.25	5.80 ± 0.20	6.20 ± 0.37	NS	NS	NS
Eosinophil (%)	2.60 ± 0.25	2.40 ± 0.25	3.00 ± 0.00	3.00 ± 0.32	NS	NS	NS
Basophil (%)	3.40 ± 0.68	2.80 ± 0.37	5.00 ± 0.00	4.40 ± 0.25	NS	NS	NS

Values are presented as means ± S.E. (n = 3). Different superscript letters refer to significant differences ($p < 0.05$). OTA: Ochratoxin-A; Hb: hemoglobin; RBCs: red blood cells; PCV: packed cell volume; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; WBCs: white blood cells; NS: not significant ($p > 0.05$).

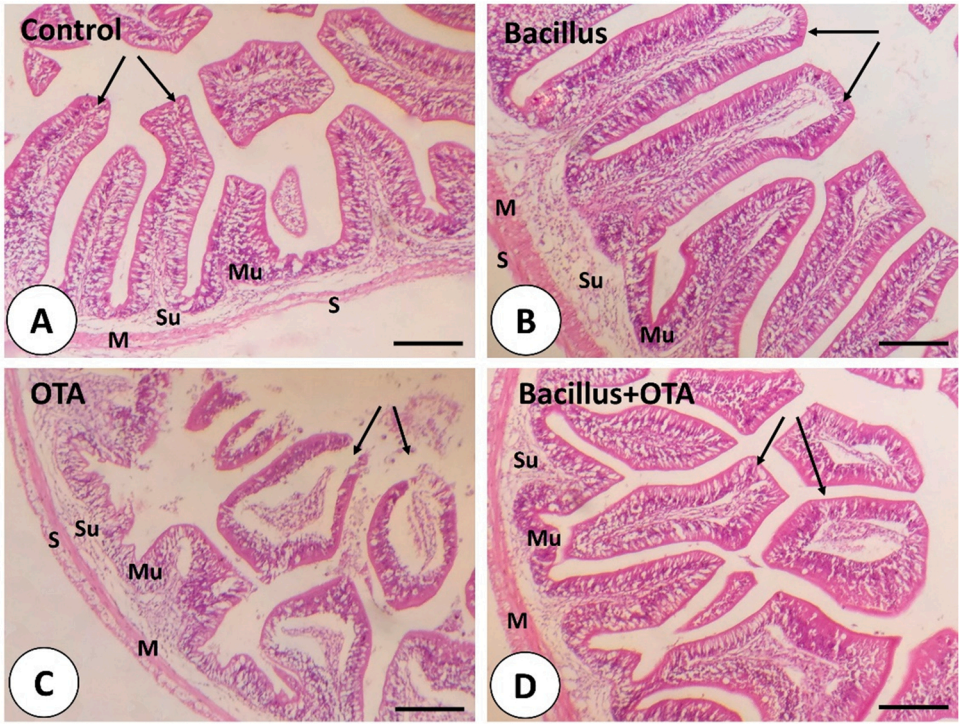

Fig. 1. Histopathological image of Nile tilapia intestine in the control (A), *Bacillus* (B), Ochratoxin-A-A (OTA) (C), and *Bacillus* + OTA-treated group. The control fish revealed normal intestinal mucosa (Mu) with intact intestinal villi (black arrow), submucosa (Su), muscularis (M) and serosa (S). The *Bacillus*-fed fish showed the same normal structure with improved appearance of intestinal villi. OTA-exposed fish showed deterioration of intestinal mucosa and stunted growth of intestinal villi (black arrow). Combination of *Bacillus* with OTA alleviated the pathological effect of OTA and improved the intestinal morphology. Stain H&E. Bar = 100 μm.

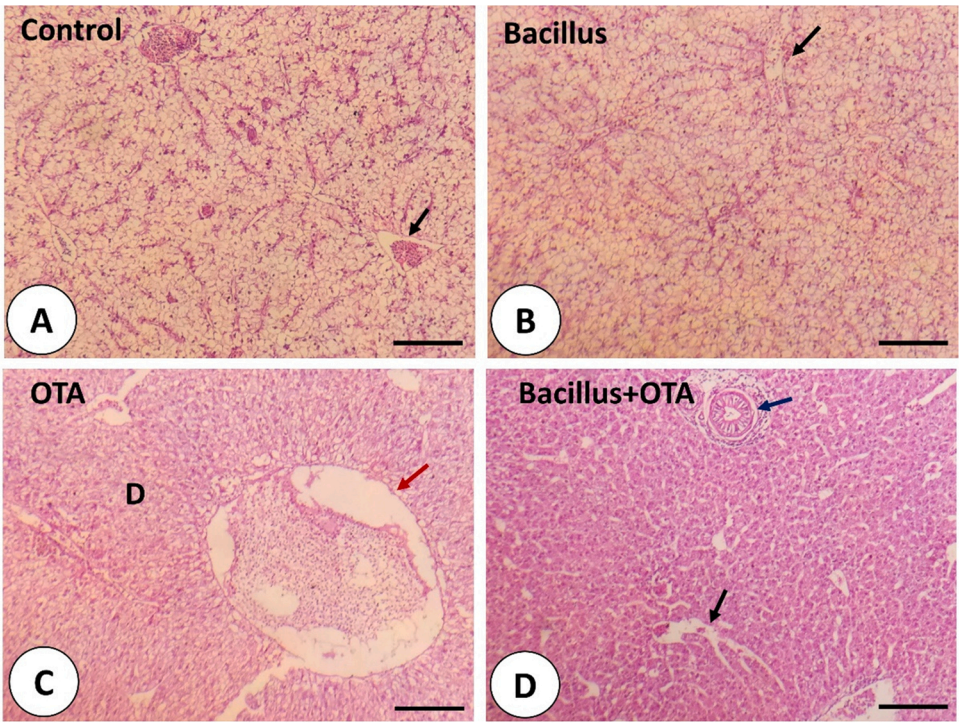

Fig. 2. Histopathological image of Nile tilapia liver in the control (A), *Bacillus* (B), Ochratoxin-A (OTA) (C), and *Bacillus* + OTA-treated group (D). liver in the control and *Bacillus* groups demonstrated normal hepatic cells and vessels (black arrow) with increased glycogen storage inside the hepatocytes. OTA group showed sever vascular dilatation and congestion (red arrow) in addition to hepatocytes degeneration (D). Addition of *Bacillus* with OTA prevented the degenerative changes and restored the normal hepatic structures with mild periductal inflammatory reaction around the bile duct (blue arrow). Stain H&E. Bar = 100 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

similar CAT, GPx, SOD, and MDA to the control. The two-way ANOVA illustrated that the BS, OTA, and BS/OTA were synergistically significant factors ($p < 0.05$) on CAT, GPx, SOD, and MDA traits (Fig. 4).

4. Discussion

Mycotoxicosis is a predominant challenge in aquafeed production with a direct effect on the aquaculture sector and an indirect effect on human health (Bernal-Algaba et al., 2021). Ochratoxin-A (OTA) contamination resulted in severe impacts on the performances of aquatic animals, such as growth retardation, impaired digestion capacity, hepato-renal malfunction, neurotoxicity, and immunosuppression (Diab et al., 2018). These negative impacts are related to the OTA protein synthesis inhibition effect through the downregulation of phenylalanine (Phe)-tRNA synthesis and reduction of the phe-hydroxylase (Ringot et al., 2006). Besides, OTA contamination induces oxidative stress and

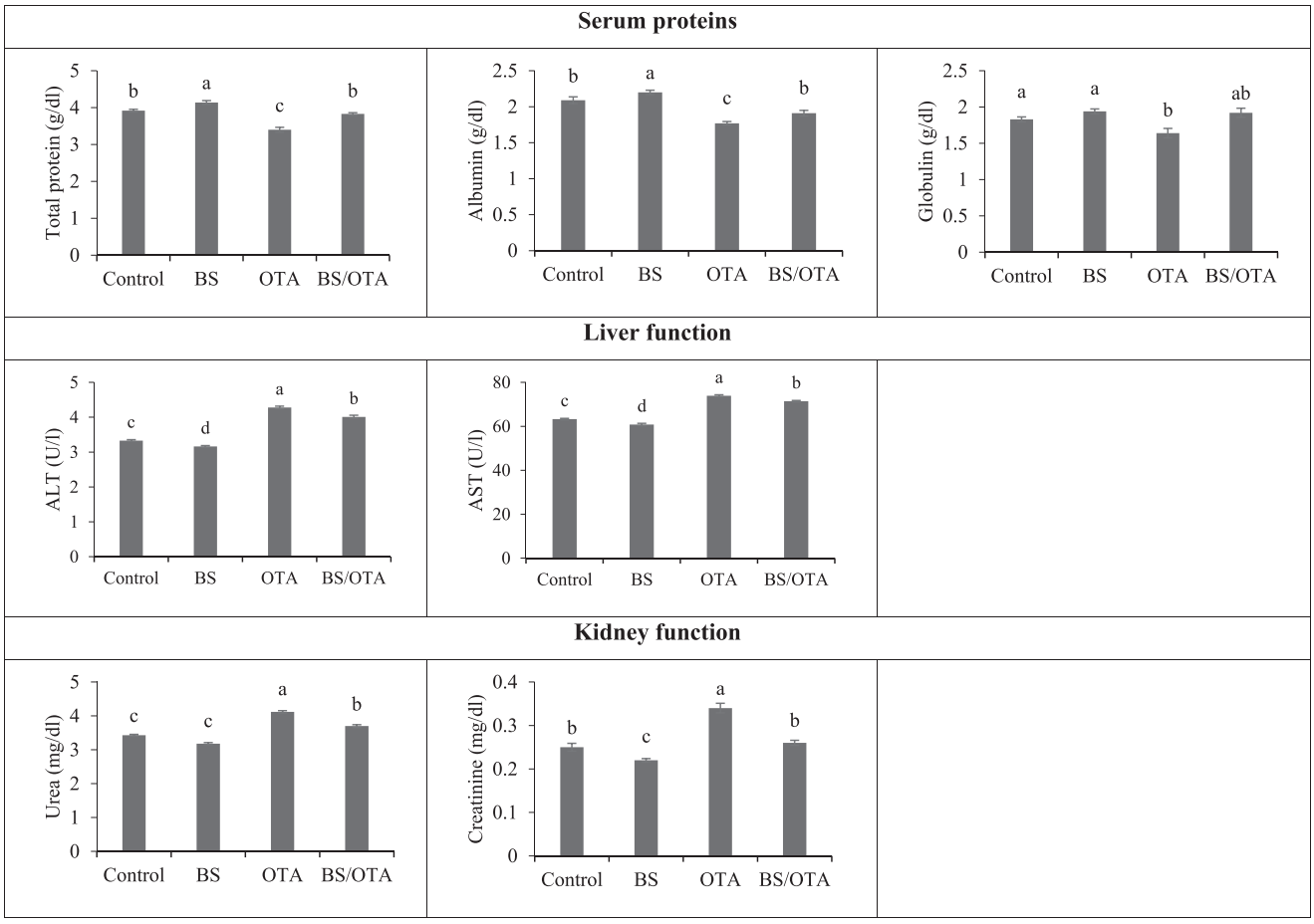

Fig. 3. Blood biochemical profile of *Liza ramada* fed diets contaminated with Ochratoxin-A (OTA) or/and *Bacillus subtilis* (BS). Bars with different letters are significant different ($p < 0.05$). ALT: alanine aminotransferase, AST: aspartate aminotransferase. Two-way ANOVA (p value) for the effects of BS, OTA, and BS×OTA = 0.001 on the biochemical profile.

results in excessive free radicals and reactive oxygen metabolites (ROS) involved in lipid peroxidation and the destruction of cellular DNA. Consequently, OTA increases apoptosis which is responsible for the programmed death of cells (Lioi et al., 2004). Nevertheless, dietary probiotics can regulate the physiological and metabolic function of a fish's entire body, leading to high resistance to OTA contamination. In this regard, Fan et al. (2018) illustrated that *Bacillus subtilis* could germinate in the GIT of Yellow River carp and result in low absorption of aflatoxin B1 (AFB1). Consequently, the low release of strain AFB1 was detected from the GIT to the bloodstream, ensuring the high detoxification role of *Bacillus* against AFB1 induced intestinal and liver damage. Further, *Bacillus* supplementation reduced AFB1 accumulation in the hepatic tissue indicating high efficacy against AFB1 toxicity (Zhang et al., 2017). Herein, we propose that *B. subtilis* may detoxify OTA-induced growth retardation and hepatic and renal failure of L. *ramada* following the finding obtained by Fan et al. (2018).

The results showed depressed growth performance in fish contaminated with OTA. Consistently, tambaqui (*Colossoma macropomum*) (Baldissera et al., 2020a, 2020b), Grass Carp (*Ctenopharyngodon idella*) (Liu et al., 2021), and Nile tilapia (*Oreochromis niloticus*) (Diab et al., 2018; Fadl et al., 2020) fed diets contaminated with OTA had impaired growth performance. The results also showed deteriorated FCR in L. *ramada* treated with OTA, which indicates the malnutrition condition of fish. The reduction in the growth performance induced by OTA is strongly related to loss of palatability, appetite, and protein synthesis disturbances (Diab et al., 2018; Zahran et al., 2016). It has been confirmed that OTA could disrupt the intestinal digestion capacity through the impairment of the intestinal barrier function (Liu et al., 2021). Accordingly, the digestibility and absorption of nutrients were impaired, which led to a reduction in metabolic function and available nutrients for cellular activity. Furthermore, digestive enzymes activity decreases with OTA contamination leading to low feed efficiency (high FCR) and growth performance. To confirm these observations, the intestinal histomorphology displayed that OTA-exposed fish showed deterioration of intestinal mucosa and stunted growth of intestinal villi. In the same line, Fadl et al. (2020) and Liu et al. (2021) stated that Nile tilapia and grass carp fed diets contaminated with OTA had distinctive intestinal histomorphological features. The authors hypothesized that intestinal impairments result from OTA's toxic effects due to the direct contact with the intestinal epithelial layer. Further, OTA induces oxidative stress and high lipid peroxidation, thereby losing intestinal function. Interestingly, the results showed that fish fed *B. subtilis* only or *B. subtilis* and OTA had high growth performance and reduced FCR. *B. subtilis* is a member of lactic acid bacteria (LAB) known for its growth-promoting efficacy, as indicated by several fish species (Di et al., 2019; Li et al., 2021; Liu et al., 2017; Tang et al., 2019; Xue et al., 2020). LAB strains can secret digestive enzyme activity and enhance the digestibility and absorption of nutrients in fish intestines and thereby high metabolic and regulated physiological function (Wang et al., 2021). LAB also can protect the intestinal barriers and balance the diversity of intestinal microorganisms with a high capacity to inhibit the abundance of pathogenic microorganisms (Cao et al., 2019). In this regard, L. *ramada* fed *B. subtilis* displayed normal intestinal structure with improved appearance of intestinal villi. Besides, a combination of *B. subtilis* with OTA alleviated the pathological effect of individual OTA on the intestinal villi and improved intestinal morphology. This may explain the

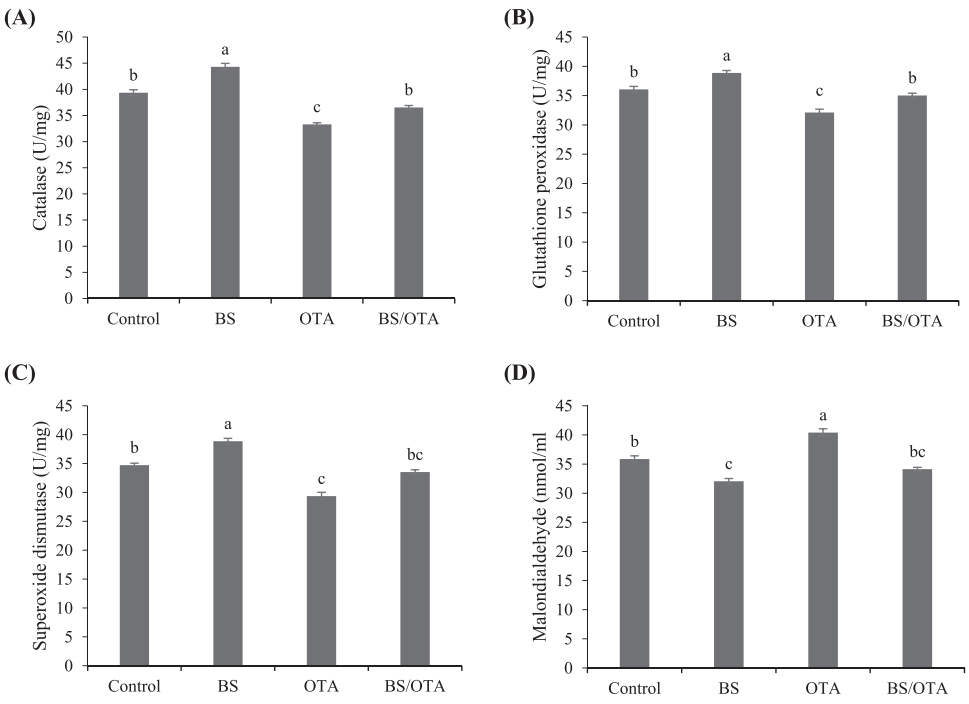

Fig. 4. Blood catalase (A), glutathione peroxidase (B), superoxide dismutase (C), and malondialdehyde (D) levels of *Liza ramada* fed diets contaminated with Ochratoxin-A (OTA) or/and *Bacillus subtilis* (BS). Bars with different letters are significant different ($p < 0.05$). Two-way ANOVA (p value) for the effects of BS, OTA, and BS×OTA = 0.001 on the catalase (A), glutathione peroxidase (B), superoxide dismutase (C), and malondialdehyde (D) levels.

improved growth performance, FCR, hepato-renal function, and antioxidative capacity in L. *ramada* fed OTA and *B. subtilis*.

The study showed high mortality in L. *ramada* treated with OTA, related to the toxic effects and weak health status. However, fish in control, *B. subtilis*, and *B. subtilis*/OTA groups had high survival rates. The lack of mortality is probably related to the improved health status and well management resulting from form *B. subtilis* supplementation. The detection of the hematological profile is required to validate the health status of fish. High hematocrit, hemoglobin, RBCS, and WBCs in the present study indicate the substantial role of *B. subtilis* in protecting L. *ramada* from the negative impacts of OTA contamination. In line with these results, channel catfish (*Ictalurus punctatus*) fed diets contaminated with OTA had reduced hematocrit levels (Manning et al., 2003).

The results also showed a reduction in the blood proteins, albumin, and globulin in L. *ramada* fed diets contaminated with OTA. Similarly, Zahran et al. (2016), Fadl et al. (2020), and Diab et al. (2018) stated that channel catfish and Nile tilapia-fed OTA had impaired blood proteins. The decrease in serum total protein is probably associated with the protein breakdown to afford enough amino acids to stimulate corticosteroid hormone. During stressful conditions, including OTA toxicity, the gluconeogenesis function is activated to produce glucose required as an energy precursor to overcome the stressful state. Further, the reduction in protein synthesis is related to the role of OTA in the inhibition of protein synthesis, as mentioned earlier. However, the inclusion of *B. subtilis* relieved the impacts of OTA on the blood proteins and resulted in high protein levels in L. *ramada*. Concurrent with these results, dietary *B. subtilis* improved blood protein levels in red seabream (*Pagrus major*) (Zaineldin et al., 2018). The role of probiotics in improving feed utilization and metabolism may affect protein synthesis and result in high protein levels in the blood.

Liver and kidney tissues are the primary targets for mycotoxicosis, and it is necessary to test their condition under OTA contamination. The results showed high ALT, AST, urea, and creatinine levels in L. *ramada* contaminated with OTA while *B. subtilis* reduced the liver (ALT and AST) and kidney (urea and creatinine) related secretions. Fadl et al. (2020) and Diab et al. (2018) reported that Nile tilapia-fed OTA displayed high ALT, AST, urea, and creatinine levels in the same line. High ALT, AST, urea, and creatinine levels indicate that OTA could induce severe hepatic and renal toxic influences. The failure of liver and kidney functions results in increased release of ALT, AST, urea, and creatinine levels in the blood. The impairment of the liver is also realized by the histological features in this study following OTA contamination. The OTA-subjected fish showed severe vascular dilatation and congestion and hepatocyte degeneration, which may explain increased ALT and AST in the blood. Similarly, Fadl et al. (2020) reported impaired liver histological features in Nile tilapia treated with OTA. High creatinine levels are generated from the breakdown of proteins in the muscles to the bloodstream then the kidney regulates its secretion (Del Zotti et al., 2008). However, high levels of secretion indicate impaired kidney function by OTA toxicity. Severe mycotoxicosis can induce inflammatory and degenerative features in perivascular and cell infiltration in the renal parenchyma (Liang et al., 2015). The inflammation in the liver and kidney tissues can also be related to the oxidative stress induced by OTA contamination. Conversely, the incorporation of *B. subtilis* regulated ALT, AST, urea, and creatinine levels in L. *ramada*, indicating the absence of liver and kidney failure. Similarly, the incorporation of probiotics in aquafeed regulated ALT, AST, urea, and creatinine levels.

Contamination with OTA is the main reason for excessive ROS production involved in the lipid peroxidation and the impairment of cellular function in the entire body of fish (Gutteridge and Halliwell, 2018). Subsequently, high oxidative stress and accumulated lipid peroxides led to DNA damage and loss of cell function (Hoehler et al., 1997), which may explain the impairment in the intestines, livers, and kidneys of L. *ramada* fed OTA in this study. Markedly, OTA resulted in high lipid peroxidation (expressed by MDA levels) and reduced antioxidative capacity (CAT, SOD, and GPx). At the same time, *B. subtilis* supplementation resulted in low MDA and high CAT, SOD, and GPx in L. *ramada*. Similarly, impaired antioxidative capacity was observed in tambaqui (Baldissera et al., 2020b) and catfish (Abdel-Wahhab et al., 2016) fed diets contaminated with OTA. Increased CAT, SOD, and GPx were also observed in grass carp (Tang et al., 2019; Xue et al., 2020; Yin et al., 2019), crucian carp (Cao et al., 2019), tongue sole (*Cynoglossus*

semilaevis) (Wang et al., 2021), olive flounder (*Paralichthys olivaceus*) (Li et al., 2021), Dabry's sturgeon (*Acipenser dabryanus*) (Di et al., 2019), and Nile tilapia (Liu et al., 2017).

5. Conclusion

In summary, OTA contamination induced growth retardation, low feed utilization, depressed antioxidative capacity, and impaired liver, kidney, and intestines in L. *ramada*. However, dietary *B. subtilis* relieved the negative impacts of OTA contamination via protecting fish's intestines, livers, and kidney function.

Funding

This work was funded by the Researchers Supporting Project (RSP-2021/36), King Saud University, Riyadh, Saudi Arabia. Chiang Mai University partially supported this research work.

CRediT authorship contribution statement

Fawzy Magouz, Hasnaa Abu-Ghanima, Mahmoud A.O. Dawood: Conceptualization. **Fawzy Magouz, Hasnaa Abu-Ghanima, Amr I. Zaineldin, Mahmoud S. Gewaily, Ali Soliman, Asem A. Amer, Eman M. Moustafa, Mahmoud A.O. Dawood**: Formal analysis. **Fawzy Magouz, Hasnaa Abu-Ghanima, Amr I. Zaineldin, Mahmoud S. Gewaily, Ali Soliman, Asem A. Amer, Eman M. Moustafa, Elsayed M. Younis, Abdel-Wahab A. Abdel-Warith, Simon J Davies, Hien Van Doan, Wanaporn Tapingkae, Mahmoud A.O. Dawood**: Funding acquisition. **Fawzy Magouz, Hasnaa Abu-Ghanima, Amr I. Zaineldin, Mahmoud S. Gewaily, Ali Soliman, Asem A. Amer, Eman M. Moustafa, Elsayed M. Younis, Abdel-Wahab A. Abdel-Warith, Simon J Davies, Hien Van Doan, Wanaporn Tapingkae, Mahmoud A.O. Dawood**: Investigation. **Fawzy Magouz, Hasnaa Abu-Ghanima, Amr I. Zaineldin, Mahmoud S. Gewaily, Ali Soliman, Asem A. Amer, Eman M. Moustafa, Mahmoud A.O. Dawood**: Methodology. **Fawzy Magouz, Eman M. Moustafa, Mahmoud A.O. Dawood**: Supervision. **Fawzy Magouz, Hasnaa Abu-Ghanima, Amr I. Zaineldin, Mahmoud S. Gewaily, Ali Soliman, Asem A. Amer, Eman M. Moustafa, Elsayed M. Younis, Abdel-Wahab A. Abdel-Warith, Simon J Davies, Hien Van Doan, Wanaporn Tapingkae, Mahmoud A.O. Dawood**: Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was funded by the Researchers Supporting Project (RSP-2021/36), King Saud University, Riyadh, Saudi Arabia. Chiang Mai University partially supported this research work.

References

Abdel-Wahhab, M.A., Aljawish, A., Kenawy, A.M., El-Nekeety, A.A., Hamed, H.S., Abdel-Aziem, S.H., 2016. Grafting of gallic acid onto chitosan nanoparticles enhances antioxidant activities in vitro and protects against ochratoxin A toxicity in catfish (*Clarias gariepinus*). Environ. Toxicol. Pharmacol. 41, 279–288.

Addo, S., Carrias, A.A., Williams, M.A., Liles, M.R., Terhune, J.S., Davis, D.A., 2017. Effects of *Bacillus subtilis* strains on growth, immune parameters, and *Streptococcus iniae* susceptibility in Nile Tilapia, *Oreochromis niloticus*. J. World Aquac. Soc. 48, 257–267.

Akinmusire, O.O., El-Yuguda, A.-D., Musa, J.A., Oyedele, O.A., Sulyok, M., Somorin, Y. M., Ezekiel, C.N., Krska, R., 2019. Mycotoxins in poultry feed and feed ingredients in Nigeria. Mycotoxin Res. 35, 149–155.

AOAC, 2012. Official Methods of Analysis of AOAC International, 19th ed. AOAC International, Gaithersburg, MD. ⟨www.eoma.aoac.org⟩.

Baldissera, M.D., Souza, C.F., da Silva, J.A., Barroso, D.C., Glória, E.M., Baldisserotto, B., Val, A.L., 2020a. Dietary exposure to ochratoxin A reduces growth performance and impairs hepatic purinergic signaling in tambaqui (*Colossoma macropomum*). Fish Physiol. Biochem. 46, 2055–2064.

Baldissera, M.D., Souza, C.F., da Silva, J.A., Barroso, D.C., Glória, E.M., Mesadri, J., Wagner, R., Baldisserotto, B., Val, A.L., 2020b. Dietary ochratoxin A (OTA) decreases growth performance and impairs muscle antioxidant system and meat fatty acid profiles in juvenile tambaqui (*Colossoma macropomum*). Comp. Biochem. Physiol. Part C Toxicol. Pharmacol. 236, 108803.

Bernal-Algaba, E., Pulgarín-Alfaro, M., Fernández-Cruz, M.L., 2021. Cytotoxicity of mycotoxins frequently present in aquafeeds to the fish cell line RTGill-W1. Toxins 13.

Bernhoft, A., Høgåsen, H.R., Rosenlund, G., Ivanova, L., Berntssen, M.H.G., Alexander, J., Eriksen, G.S., Fæste, C.K., 2017. Tissue distribution and elimination of deoxynivalenol and ochratoxin A in dietary-exposed Atlantic salmon (*Salmo salar*). Food Addit. Contam. Part A 34, 1211–1224.

Brown, K.A., Mays, T., Romoser, A., Marroquin-Cardona, A., Mitchell, N.J., Elmore, S.E., Phillips, T.D., 2014. Modified hydra bioassay to evaluate the toxicity of multiple mycotoxins and predict the detoxification efficacy of a clay-based sorbent. J. Appl. Toxicol. 34, 40–48.

Bui-Klimke, T.R., Wu, F., 2015. Ochratoxin A and human health risk: a review of the evidence. Crit. Rev. Food Sci. Nutr. 55, 1860–1869.

Cao, H., Yu, R., Zhang, Y., Hu, B., Jian, S., Wen, C., Kajbaf, K., Kumar, V., Yang, G., 2019. Effects of dietary supplementation with β-glucan and *Bacillus subtilis* on growth, fillet quality, immune capacity, and antioxidant status of Pengze crucian carp (*Carassius auratus* var. Pengze). Aquaculture 508, 106–112.

Collier, H.B., 1944. Standardization of blood haemoglobin determinations. Can. Med. Assoc. J. 50, 550–552.

Dacie, J.V., Lewis, S.M., 1995. Practical haematology. In: Practical Hæmatology, pp. 609–609.

Dawood, M.A.O., 2021. Nutritional immunity of fish intestines: important insights for sustainable aquaculture. Rev. Aquac. 13, 642–663.

Del Zotti, F., Visonà, E., Massignani, D., Abaterusso, C., Lupo, A., Gambaro, G., 2008. General practitioners' serum creatinine recording styles. J. Nephrol. 21, 106–109.

Di, J., Chu, Z., Zhang, S., Huang, J., Du, H., Wei, Q., 2019. Evaluation of the potential probiotic *Bacillus subtilis* isolated from two ancient sturgeons on growth performance, serum immunity and disease resistance of *Acipenser dabryanus*. Fish Shellfish Immunol. 93, 711–719.

Diab, A.M., S, R.M., Abeer, E.-K.M.S., Ali, G.I.E., El-Habashi, N., 2018. Experimental ochratoxicosis A in Nile tilapia and its amelioration by some feed additives. Int. J. Vet. Sci. Med. 6, 149–158.

Doumas, B.T., Bayse, D.D., Carter, R.J., Peters, T., Schaffer, R., 1981. A candidate reference method for determination of total protein in serum. I. Development and validation. Clin. Chem. 27, 1642–1650.

Du, R.-Y., Zhang, H.-Q., Chen, J.-X., Zhu, J., He, J.-Y., Luo, L., Lin, S.-M., Chen, Y.-J., 2021. Effects of dietary *Bacillus subtilis* DSM 32315 supplementation on the growth, immunity and intestinal morphology, microbiota and inflammatory response of juvenile largemouth bass *Micropterus salmoides*. Aquac. Nutr. (n/a).

Dumas, B.T., Biggs, H.G., 1972. In: Cooper, Gerald R. (Ed.), Standard methods of clinical chemistry. Academic Press, New York, pp. 1–333. https://www.sciencedirect.com/bookseries/standard-methods-of-clinical-chemistry/vol/7/suppl/C.

El-Saadony, M.T., Alagawany, M., Patra, A.K., Kar, I., Tiwari, R., Dawood, M.A.O., Dhama, K., Abdel-Latif, H.M.R., 2021. The functionality of probiotics in aquaculture: an overview. Fish Shellfish Immunol. 117, 36–52.

Fadl, S.E., El-Shenawy, A.M., Gad, D.M., El Daysty, E.M., El-Sheshtawy, H.S., Abdo, W.S., 2020. Trial for reduction of Ochratoxin A residues in fish feed by using nanoparticles of hydrated sodium aluminum silicates (NPsHSCAS) and copper oxide. Toxicon 184, 1–9.

Fan, Y., Liu, L., Zhao, L., Wang, X., Wang, D., Huang, C., Zhang, J., Ji, C., Ma, Q., 2018. Influence of *Bacillus subtilis* ANSB060 on growth, digestive enzyme and aflatoxin residue in Yellow River carp fed diets contaminated with aflatoxin B1. Food Chem. Toxicol. 113, 108–114.

Gewaily, M.S., Abumandour, M.M., 2021. Gross morphological, histological and scanning electron specifications of the oropharyngeal cavity of the hooded crow (*Corvus cornix* pallescens). Anat. Histol. Embryol. 50, 72–83.

Glencross, B.D., Baily, J., Berntssen, M.H.G., Hardy, R., MacKenzie, S., Tocher, D.R., 2020. Risk assessment of the use of alternative animal and plant raw material resources in aquaculture feeds. Rev. Aquac. 12, 703–758.

Gonçalves, R.A., Schatzmayr, D., Albalat, A., Mackenzie, S., 2020. Mycotoxins in aquaculture: feed and food. Rev. Aquac. 12, 145–175.

Gutteridge, J.M.C., Halliwell, B., 2018. Mini-Review: oxidative stress, redox stress or redox success? Biochem. Biophys. Res. Commun. 502, 183–186.

Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-Transferases: the first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249, 7130–7139.

Hathout, A.S., Abel-Fattah, S.M., Abou-Sree, Y.H., Fouzy, A.S.M., 2020. Incidence and exposure assessment of aflatoxins and ochratoxin A in Egyptian wheat. Toxicol. Rep. 7, 867–873.

Hoehler, D., Marquardt, R.R., Rohlich, A.A.F., 1997. Lipid peroxidation as one mode of action in ochratoxin A toxicity in rats and chicks. Can. J. Anim. Sci. 77, 287–292.

Houston, A., 1990. Blood and circulation/Methods for fish biology. Amer. Fish. Society, NY.

Kari, Z.A., Kabir, M.A., Dawood, M.A.O., Razab, M.K.A.A., Ariff, N.S.N.A., Sarkar, T., Pati, S., Edinur, H.A., Mat, K., Ismail, T.A., Wei, L.S., 2021. Effect of fish meal substitution with fermented soy pulp on growth performance, digestive enzyme, amino acid profile, and immune-related gene expression of African catfish (*Clarias gariepinus*). Aquaculture, 737418.

Klontz, G., 1994. Fish hematology. Tech. Fish Immunol. 3, 121–131.
Li, Y., Yang, Y., Song, L., Wang, J., Hu, Y., Yang, Q., Cheng, P., Li, J., 2021. Effects of dietary supplementation of *Lactobacillus plantarum* and *Bacillus subtilis* on growth performance, survival, immune response, antioxidant capacity and digestive enzyme activity in olive flounder (*Paralichthys olivaceus*). Aquac. Fish. 6, 283–288.
Liang, R., Shen, X.L., Zhang, B., Li, Y., Xu, W., Zhao, C., Luo, Y., Huang, K., 2015. Apoptosis signal-regulating kinase 1 promotes Ochratoxin A-induced renal cytotoxicity. Sci. Rep. 5, 1–12.
Lioi, M.B., Santoro, A., Barbieri, R., Salzano, S., Ursini, M.V., 2004. Ochratoxin A and zearalenone: a comparative study on genotoxic effects and cell death induced in bovine lymphocytes. Mutat. Res. Genet. Toxicol. Environ. Mutagen. 557, 19–27.
Liu, H., Wang, S., Cai, Y., Guo, X., Cao, Z., Zhang, Y., Liu, S., Yuan, W., Zhu, W., Zheng, Y., Xie, Z., Guo, W., Zhou, Y., 2017. Dietary administration of *Bacillus subtilis* HAINUP40 enhances growth, digestive enzyme activities, innate immune responses and disease resistance of tilapia, *Oreochromis niloticus*. Fish Shellfish Immunol. 60, 326–333.
Liu, X., Wu, P., Jiang, W.-D., Liu, Y., Jiang, J., Kuang, S.-Y., Tang, L., Zhou, X.-Q., Feng, L., 2021. Effects of dietary Ochratoxin A on growth performance and intestinal apical junctional complex of juvenile grass carp (*Ctenopharyngodon idella*). Toxins 13.
Logan, N.A., Halket, G., 2011. Developments in the taxonomy of aerobic, endospore-forming bacteria. In: Logan, N.A., Vos, P. (Eds.), Endospore-forming Soil Bacteria. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 1–29.
Manning, B.B., Ulloa, R.M., Li, M.H., Robinson, E.H., Rottinghaus, G.E., 2003. Ochratoxin A fed to channel catfish (*Ictalurus punctatus*) causes reduced growth and lesions of hepatopancreatic tissue. Aquaculture 219, 739–750.
McCord, J.M., Fridovich, I., 1969. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). J. Biol. Chem. 244, 6049–6055.
Meucci, V., Armani, A., Tinacci, L., Guardone, L., Battaglia, F., Intorre, L., 2021. Natural occurrence of ochratoxin A (OTA) in edible and not edible tissue of farmed gilthead seabream (*Sparus aurata*) and European seabass (*Dicentrarchus labrax*) sold on the Italian market. Food Control 120, 107537.
Pietsch, C., 2020. Risk assessment for mycotoxin contamination in fish feeds in Europe. Mycotoxin Res. 36, 41–62.
Pinheiro, R., Rodrigues, A., Lima, C.E., Santos, J., Pereyra, C.M., Torres, A.M., Cavaglieri, L.R., Lopes, J., Muratori, M., 2020. *Saccharomyces cerevisiae* as a probiotic agent and a possible aflatoxin B_1 adsorbent in simulated fish intestinal tract conditions. Arq. Bras. Med. Veter. Zootec. 72, 862–870.
Ringot, D., Chango, A., Schneider, Y.-J., Larondelle, Y., 2006. Toxicokinetics and toxicodynamics of ochratoxin A, an update. Chem. Biol. Interact. 159, 18–46.
Shukry, M., Albogami, S., Gewaily, M., Amer, A.A., Soliman, A.A., Alsaiad, S.M., El-Shehawi, A.M., Dawood, M.A.O., 2021. Growth performance, antioxidative capacity, and intestinal histomorphology of Grey Mullet (*Liza ramada*)–fed dietary zinc nanoparticles. Biol. Trace Elem. Res.
Tang, Y., Han, L., Chen, X., Xie, M., Kong, W., Wu, Z., 2019. Dietary supplementation of probiotic *Bacillus subtilis* affects antioxidant defenses and immune response in grass carp under *Aeromonas hydrophila* Challenge. Probiotics Antimicrob. Proteins 11, 545–558.
Tschirren, L., Siebenmann, S., Pietsch, C., 2018. Toxicity of ochratoxin to early life stages of zebrafish (*Danio rerio*). Toxins 10.
Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86, 271–278.
Wang, Y., Wang, Q., Xing, K., Jiang, P., Wang, J., 2021. Dietary cinnamaldehyde and *Bacillus subtilis* improve growth performance, digestive enzyme activity, and antioxidant capability and shape intestinal microbiota in tongue sole, *Cynoglossus semilaevis*. Aquaculture 531, 735798.
Wintrobe, M.M., 1934. Variations in the size and hemoglobin content of erythrocytes in the blood of various vertebrates. Folia Haematol. 51, 32–49.
Wu, T.-S., Lin, Y.-T., Huang, Y.-T., Yu, F.-Y., Liu, B.-H., 2020. Ochratoxin A triggered intracerebral hemorrhage in embryonic zebrafish: involvement of microRNA-731 and prolactin receptor. Chemosphere 242, 125143.
Xue, J., Shen, K., Hu, Y., Hu, Y., Kumar, V., Yang, G., Wen, C., 2020. Effects of dietary *Bacillus cereus*, B. *subtilis*, *Paracoccus marcusii*, and *Lactobacillus plantarum* supplementation on the growth, immune response, antioxidant capacity, and intestinal health of juvenile grass carp (*Ctenopharyngodon idellus*). Aquac. Rep. 17, 100387.
Yin, Y., Yue, X., Zhang, D., Zhang, P., Abdallah, A., Yin, Y., Cai, Y., Li, Y., 2019. Study of bioaccumulation, hematological parameters, and antioxidant responses of *Carassius auratus gibelio* exposed to dietary lead and *Bacillus subtilis*. Biol. Trace Elem. Res. 189, 233–240.
Zahran, E., Manning, B., Seo, J.-K., Noga, E.J., 2016. The effect of Ochratoxin A on antimicrobial polypeptide expression and resistance to water mold infection in channel catfish (*Ictalurus punctatus*). Fish Shellfish Immunol. 57, 60–67.
Zahran, E., Risha, E., Hamed, M., Ibrahim, T., Palić, D., 2020. Dietary mycotoxicosis prevention with modified zeolite (Clinoptilolite) feed additive in Nile tilapia (*Oreochromis niloticus*). Aquaculture 515, 734562.
Zaineldin, A.I., Hegazi, S., Koshio, S., Ishikawa, M., Bakr, A., El-Keredy, A.M.S., Dawood, M.A.O., Dossou, S., Wang, W., Yukun, Z., 2018. *Bacillus subtilis* as probiotic candidate for red sea bream: growth performance, oxidative status, and immune response traits. Fish Shellfish Immunol. 79, 303–312.
Zhang, L., Ma, Q., Ma, S., Zhang, J., Jia, R., Ji, C., Zhao, L., 2017. Ameliorating effects of *Bacillus subtilis* ANSB060 on growth performance, antioxidant functions, and aflatoxin residues in ducks fed diets contaminated with aflatoxins. Toxins 9.