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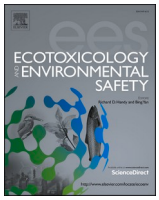

Potential effect dietary supplementation of calcium tetraborate in quails exposed to cadmium: Its impact on productive performance, oxidative stress, cecal microflora, and histopathological changes

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ABSTRACT

Cadmium (Cd) is a ubiquitous environmental pollutant, and Cd exposure harms human health, agriculture, and animal husbandry. The present study aimed to investigate the potential protective effect of dietary supplementation of calcium tetraborate (CTB) on productive performance, oxidative stress, cecal microflora, and histopathological changes in quail exposed to Cd. A total of one hundred twenty, 6-week-old Japanese quail (four females and two males/replicate) were divided into four groups (30 quails/group): the control group (feeding basic diet), CTB group (basic diet containing 300 mg/kg CaB_4O_7, 22.14% elemental B/kg diet), the Cd group (basic diet containing 100 mg/kg cadmium chloride ($CdCl_2$) (total Cd content of 92.1 mg/kg)) and the CTB + Cd group (basic diet containing 300 mg/kg CTB and 100 mg/kg $CdCl_2$). The results showed that Cd exposure caused decreased performance, increased the proportion of broken and soft-shelled eggs, induced oxidative stress, affected cecal microflora, epicardial hemorrhages in the heart, focal necrosis in the liver, degeneration in the kidneys, and degenerated and necrotic seminiferous tubules in the testicles. CTB prevented Cd-induced oxidative stress in liver tissue by increasing total antioxidant status and reducing total oxidant status. In addition, CTB improved egg production and feed conversion ratio (FCR). CTB protected the cecal microflora by inhibiting *Enterobacteriaceae* and promoting *Lactobacillus*. CTB also reduced Cd-induced histopathological damage in the heart, liver, kidneys, and testicles. In conclusion, these findings suggest that CTB could be used in Cd-challenged quail, and this compound provides new insights into the toxicity of environmental Cd.

1. Introduction

Cadmium (Cd) is a toxic heavy metal that harms human and animal health (Bi et al., 2021). Cd is non-biodegradable and has high environmental persistence and a high soil-to-plant transfer rate (Zhang et al., 2021). Cd, a significant industrial raw material, is extensively utilized in various industrial endeavors, such as manufacturing alloys, pigments, and batteries (Abdallah and El-Refaei, 2022). In nature, Cd is formed through volcanic activities, forest fires, and soil particles carried by wind. However, Cd contaminates water, soil, and plants due to the incineration of waste, mining, combustion of fossil fuels, and using phosphate fertilizers and Cd-containing sludges (Zhang et al., 2021). Some agricultural practices can elevate the deposition of Cd in the soil (Hafez et al., 2023). Human populations and animals are exposed to Cd through air, drinking water, and food contaminants. Cd can accumulate in organisms through the food chain (Bi et al., 2021). The kidney, liver, pancreas, reproductive system, and neurological system were among the many organs of animals damaged by cadmium toxicity (Zhang et al.,

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2021). Exposure to Cd in livestock, including poultry, poses health risks and harms animal production. It can lead to reduced growth performance and hinder feed utilization efficiency, impacting overall productivity and economic outcomes in the livestock industry (Khafaga et al., 2019).

Cd can induce the production of reactive oxygen species (ROS), leading to cellular damage by depleting enzyme activities through lipid peroxidation and interactions with nuclear proteins and DNA (Abdallah and El-Refaei, 2022). The cumulative impact of this heavy metal is likely related to the action of metallothionein (Zhu et al., 2020; Hafez et al., 2023). Cd toxicity arises from the competition between Cd and essential metals, leading to alterations in metal membrane transport and energy or mitochondrial dysfunction and injury resulting from its binding to thiol groups (Elgharib et al., 2022; Unsal et al., 2020; Okutu Jackson and Onitsha Enebrayi, 2022). Additionally, Cd induces protein structural disruption by binding to sulfhydryl groups. The liver serves as the primary organ for xenobiotic metabolism and detoxification; however, the presence of Cd on hepatic cell membranes suppresses the activity of hepatic antioxidant enzymes while also generating ROS (Noor et al., 2022; Elgharib et al., 2022). The dietary nutrient composition also influences Cd concentration in birds' tissues. For instance, diets with high calcium (Ca) content can hinder the absorption of Cd from gastrointestinal tracts (Kar and Patra, 2021). Ca plays a critical role in many essential cellular functions. Ca is a central cell signaling molecule that generates membrane potentials and electrical signals. It does this by acting as a second messenger and contributing to electrochemical change (Cooper and Dimri, 2023). Cd has a similar physicochemical structure to Ca, so cells can take Cd instead of Ca. When Cd displaces Ca or disrupts Ca-mediated signaling pathways, this can lead to decreased intracellular calcium levels and Cd replacing the normal functions of Ca (Choong et al., 2014). The toxicity of Cd accumulated in the body is closely associated with oxidative stress. Therefore, chemicals with antioxidant capacity and enhancing the antioxidant enzymatic defense system can counteract Cd's toxicity (Chen et al., 2022). (Chen et al., 2022).

In recent years, significant attention has been focused on elucidating the pleiotropic effects of boron, which encompass its ability to activate immune responses, engage in antioxidant activities, influence the metabolism of bones, enhance animal performance, and modulate various body systems. Moreover, boron has the potential to exert an impact on the metabolism of enzymes and minerals. Boron indirectly influences the metabolism of Ca, phosphorus, and magnesium in animals by modulating the hormone or enzyme systems (Abdelnour et al., 2018). Boric acid may act as a chelating agent that can bind heavy metals such as Cd. This means that boric acid can reduce the circulation and tissue accumulation of Cd in the body. In this way, boric acid can reduce the penetration and toxicity of Cd into the body (Kar and Patra, 2021). In numerous studies, boron has demonstrated antioxidant properties (Coban et al., 2015) and hepatoprotective effects (Ince et al., 2014). A study conducted by Ince et al. (2014) proposed that boron reduces oxidative damage by enhancing the body's glutathione reserves and inhibiting other ROS (Kar and Patra, 2021). At the gut and tissue levels, many minerals interact with each other, which can influence their absorption and utilization. Boron and Ca have been investigated for their potential to mitigate Cd toxicity (Poirier et al., 1983; Martin et al., 2006; Babaknejad et al., 2018). However, the available data concerning their effects on poultry still needs to be improved. In laying hens, adding dietary boron at different levels (0, 60, and 120 mg/kg) did not alleviate the adverse impact of dietary Cd exposure on the performance and eggshell quality (Olgun, 2015).

Based on the literature, dietary supplementation of calcium tetraborate (CaB_4O_7)(CTB) may provide new insight into quails exposed to Cd, mainly through its antioxidative properties. This study aimed to investigate the effect of dietary supplementation of Cd on productive performance, oxidative stress, cecal microflora, and histopathological changes in quail and whether these effects could be attenuated with CTB supplementation to the diet.

2. Materials and methods

2.1. Preparation and characterization of calcium tetraborate

As a powder form, calcium tetraborate (CTB-CaB_4O_7) was synthesized by a high-temperature solid-state reaction method. For the synthesis of the CTB, initial materials are calcium carbonate (98.5% pure, Merck), boric acid (99.5% pure, Merck), and urea (99.5% pure, Merck). The obtained CTB powders were characterized by the X-ray diffractometer (XRD) analysis method. The CTB compound was analyzed using Rigaku MiniFlex XRD with Cu-Kα radiation source (λ = 1,54056 Å). The scanning speed was 2°/min, and the 2θ range was between 3° and 90°. Rigaku MiniFlex XRD device with Cu-Kα line (λ = 1.54056 Å) was used as the radiation source. The scanning speed was 2°/min, and the 2θ range was between 3° and 60°. The XRD of CTB is displayed in Fig. 1. According to the XRD results in Fig. 1, the most intense peaks in the diffractogram overlap with the JCPDS (Card No: 00–031-0253) card. The CTB compound has been successfully synthesized (Fig. 1)(İflazoglu et al., 2018).

2.2. Experimental design, animals and diet

A total of one hundred twenty, 6-week-old Japanese quail (Coturnix japonica) were randomly divided into four treatments with five replicates (four females and two males/replicate) according to average body weight and laying rate. The quails (total number of 120) were divided

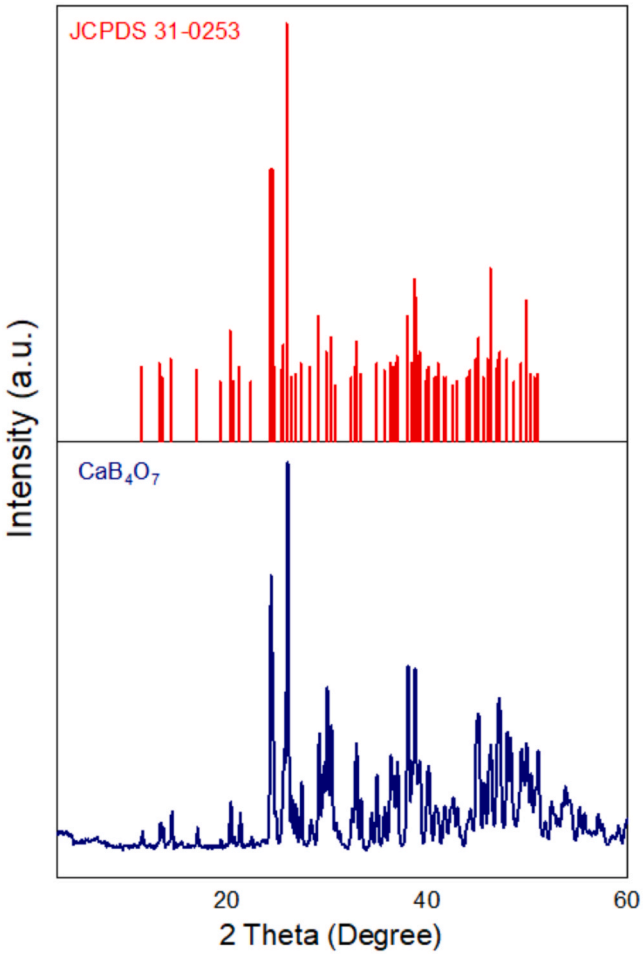

Fig. 1. XRD from calcium tetraborate and matched PDF card (00–031-0253).

into four groups (30 quails/group): the control group, the calcium tetraborate (CTB) group (300 mg/kg CaB_4O_7, 22.14% elemental B/kg diet), the Cd group (100 mg/kg cadmium chloride ($CdCl_2$) (total Cd content of 92.1 mg/kg)) and the CTB + Cd group (300 mg/kg CTB and 100 mg/kg $CdCl_2$). The quails were on a 16 L:8D lighting schedule, and the experiment duration was eight weeks. The quails were given ad libitum access to feed and water. The doses of Cd and CTB used in the experiment were administered based on previous studies and other studies on Japanese quails (Olgun et al., 2009; Kar and Patra, 2021). The diets were formulated using NRC (1994) guidelines to fulfill the nutritional needs of Japanese quail (Table 1). The basal diet was analyzed by AOAC (2000) to determine the composition of the diet. Crude fiber was determined by the Crampton and Maynard (1938) method, and metabolizable energy was estimated using the equation of Carpenter and Clegg (1956). The CTB and $CdCl_2$ were added to the diet and thoroughly mixed weekly. Following this, they were stored in dark bags in dry environments until they were used to feed Japanese quail. The $CdCl_2$ was obtained by Sigma-Aldrich (Cadmium chloride anhydrous). All animal experimental procedures were carried out according to the Animal Experiments Local Ethics Committee guidelines at Firat University (2020/11).

2.3. Determination of productive performance

To determine the body weight change, the body weight of each quail was individually recorded at the end and the beginning of the experiment. The feed intake was recorded based on replicates each week. During the experimental period, egg numbers and weight per replicate were recorded daily. Feed conversion ratio (FCR) (g feed/g egg) was calculated as grams of total feed intake per replicate per gram of total egg mass per replicate. Each replicate's average feed intake, egg weight, egg production, and FCR were calculated at 4-week intervals. The number of broken, soft-shelled, and extra-small eggs was determined from the eggs recorded. The percentage of the number of abnormal eggs (broken, soft-shelled, and extra-small) was determined by proportioning this number to the total number of eggs produced. The mortality of quails was tracked during the trial period.

Table 1
Composition of experimental diet (%)[a].

Ingredients	%	Nutritional level	Value
Maize	56.00	Dry matter %	90.50
Soybean meal (44% CP)	26.80	Crude protein %	17.50
Sunflower meal (28% CP)	1.20	Crude cellulose %	3.65
Wheat bran	2.10	Ether extract %	4.00
Sunflower oil	2.35	Crude ash %	13.58
Sodium chloride	0.35	B %	0.125
L - Lysine hydrochloride	0.15	Cd %	ND
L - Treonine	0.10	Ca %	6.335
Sodium bicarbonate	0.20	Mg %	0.280
DL - Methionine	0.10	Na %	0.428
Vitamin - Mineral premix	0.35	Fe %	0.023
Ground limestone	8.00	K %	0.919
Dicalcium phosphate	2.30	Mn %	0.016
Total	100	Cu %	0.001
		Zn %	0.020
		Phosphorus[b]	0.35
		Lysine[b]	1.00
		Threonine[b]	0.74
		ME, kcal/kg[b]	2750

[a] CaB_4O_7 (300 mg CaB_4O_7 per kg diet) was added to the basal diet.
[b] Vitamin-mineral premix (per 1 kg): vitamin A, 8000 IU; vitamin D3, 3000 IU; vitamin E, 25 IU; menadione, 1.5 mg; vitamin B12, 0.02 mg; biotin, 0.1 mg; folacin, 1 mg; niacin, 50 mg; pantothenic acid, 15 mg; pyridoxine, 4 mg; riboflavin, 10 mg; and thiamin, 3 mg copper (copper sulphate), 10 mg; iodine (ethylenediamine dihydriodide), 1.0 mg; iron (ferrous sulphate monohydrate), 50 mg; manganese (manganese sulphate monohydrate), 60 mg; and zinc (zinc sulphate monohydrate), 60 mg, selenium (sodium selenite), 0.42 mg. ND: Not detected; Detection limit of: Cd < 0.0001. [c]Calculated.

2.4. Sample collection

At the end of the experiment (8 weeks), ten quails (five females and five males) from each group were sacrificed by cervical dislocation. Then, the heart, liver, kidney, testicles, and cecum of quails were stockpiled at − 80 ℃ for future analysis.

2.5. Microbiological analyses of cecal samples

The cecum was aseptically excised, and the samples were aseptically transferred into sterile plastic tubes, then promptly placed within labeled containers filled with ice for immediate transportation to the laboratory for analysis of cecal microbial populations. To conduct microbiological analyses, 1 g of the cecal content was diluted with 9 mL of 0.1% sterile peptone water in a sterile stomacher bag homogenized for 1 min on a bagmixer (BagMixer Interscience, France). Following homogenization, decimal dilutions ranging from 10^{-1} to 10^{-5} were prepared. Diluted samples (1 mL) were inoculated into selective agar for bacterial enumeration. The microbial populations of the cecal samples were analyzed for total viable count (TVC), *Lactobacillus* spp., *Enterobacteriaceae*, *coliforms*, and *E. coli*. For this purpose, the TVC (35 ± 1 °C for 48 h) (USDA/FSIS, 2015), *Lactobacillus* spp. (30 ± 1 °C for three days) (ISO 4, 1521, 2021), *Enterobacteriaceae* (37 ± 1 °C for 24 h) (ISO, 21528–2, 2017), *coliforms* (37 ± 1 °C for 24 h) (ISO, 4832, 2021), *E. coli* (2 h at 37 ± 1 °C, then 22 h at 44.5 ± 0.5 °C) (ISO 16649–2, 2021) were determined by using plate count agar, and de Mann Rogosa Sharpe, violet red bile dextrose, violet red bile lactose, tryptone bile x-glucuronide agars (Merck, Darmstad, Germany), respectively. After incubation counting, the bacteria colonies on each plate were counted, and the results were expressed as $\log_{10}$ CFU/g cecal content.

2.6. Determination of oxidant/antioxidant indexes in liver samples

2.6.1. Total antioxidant status (TAS)

TAS levels were measured with commercially available kits (Relassay, Turkey). The novel automated method relies on fading the characteristic color of a more stable 2,2′-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) radical cation by antioxidants. The assay demonstrates excellent precision with values below 3%. Results were expressed as mmol Trolox equivalent/L (Erel, 2004).

2.6.2. Total oxidant status (TOS)

TOS levels were assessed using Relassay kits in Turkey. In this novel method, sample oxidants converted the ferrous ion-o-dianisidine complex into a ferric ion. Glycerol in the medium enhanced this oxidation process. Ferric ions formed a colored complex with xylenol orange under acidic conditions. The color intensity, measurable via spectrophotometry, corresponded to the total oxidant molecules in the sample. Calibration was performed using hydrogen peroxide, and results were reported as μmol H_2O_2 equivalent/L (Erel, 2005).

2.6.3. Oxidative stress index (OSI)

The OSI was determined as the ratio of TOS to TAS. To calculate it, the TAS unit was converted to μmol/L, and the OSI value was calculated according to Harma et al. (2003), Kosecik et al. (2005), and Yumru et al. (2009).

2.7. Histopathological analysis

At the end of the experimental period, the heart, liver, kidney, and testicles of the decapitated quails were removed and fixed in a 10% buffered formaldehyde solution for histopathological examination. After approximately 48 h of fixation, routine tissue processing (Leica TP 1020, Wetzlar, Germany) and paraffin blocks (Leica EG 1150 H, Wetzlar, Germany) were prepared. Histological sections from these blocks were taken with a rotary microtome (Leica RM2125, Wetzlar, Germany) and

stained with the hematoxylin-eosin method (Luna, 1968) (Leica Autostainer XL, Wetzlar, Germany) and examined under a light microscope (Olympus BX43, Tokyo, Japan). Selected sections were photographed microscopically (Olympus DP72, Tokyo, Japan).

2.8. Statistical analysis

Levene's test determined homogeneity of variance, and normal distribution was verified by the Shapiro–Wilk test. The data were collected with the SPSS 22.0 software (SPSS Incorporated, USA) and were analyzed using one-way ANOVA with Tukey post hoc test. Chi-square analysis was used to determine differences in survival between groups. All experimental data were expressed as mean ± standard error of the mean (SEM), and it was considered that $P < 0.05$ represents statistically significant.

3. Results

3.1. Productive performance

The impact of Cd and CTB on the productive performance of laying Japanese quails is presented in Table 2. No significant difference was found in egg weight and feed intake (days 1 to 28). Cd-induced a notable decrease in the productive performance of quails. The FCR was increased significantly ($P < 0.001$) at 100 mg Cd/kg feed doses during the experimental period. During days 1–56, the egg production rate in the Cd supplementation was decreased by 32.8% ($P < 0.001$), while the FCR was increased by 58.3% ($P < 0.001$) compared with the control group. Cd supplementation in the diet decreased egg weight ($P < 0.01$), feed intake ($P < 0.01$), and egg production ($P < 0.001$) and increased FCR ($P < 0.001$)on days 1 to 56. Dietary CTB supplementation improved egg production and decreased FCR compared to the Cd group. Egg weight, feed intake, egg production, and FCR had no significant difference between the control and CTB groups ($P > 0.05$). In the CTB+Cd group, the egg production was significantly higher than those in the Cd group and substantially lower than those in the CTB group ($P < 0.001$).

The control and CTB quail had a lower mortality rate (Fig. 2). Cd supplementation increased the mortality rate in both Cd and CTB+Cd groups compared to the control group ($P > 0.05$; Fig. 2). However, there was no statistically significant difference in mortality among the groups.

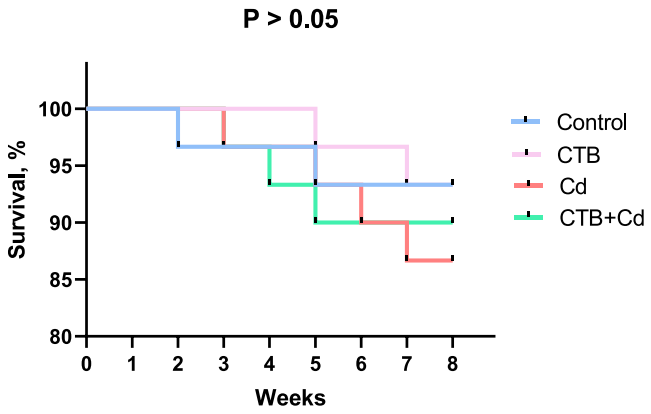

Fig. 2. Effect of CTB and/or Cd on survival rate.

The effects of dietary supplementation of CTB on body weight and the percentage of abnormal eggs (broken, soft-shelled, and extra-small) of quail exposed to Cd are presented in Table 3. The final body weight of the Cd group was significantly reduced compared to the control group ($P < 0.05$). The ratio of broken and soft-shelled eggs increased with Cd supplementation ($P < 0.01$).

3.2. Cecal microflora

The effects of dietary CTB on the cecal microflora of laying quail-fed diet contaminated with Cd are shown in Fig. 3. The *E. coli* in CTB and CTB+Cd groups were significantly lower than in the control group ($P < 0.05$). The *Enterobacteriaceae* increased significantly with Cd supplementation relative to the control and CTB ($P < 0.05$). However, the *Lactobacillus* population was more significant in the cecal of quail fed with the control and CTB diets than the Cd diet ($P < 0.01$).

3.3. Liver antioxidant parameters

The effects of CTB supplementation and Cd challenge on liver TAS, TOS, and OSI levels in quails are presented in Fig. 4. The level of TOS in the liver of the Cd group was significantly increased compared with the control ($P < 0.01$). The supplementation of CTB of quail exposed to Cd caused a decline (by 19%) in the liver TOS compared to the Cd group. The supplementation of Cd decreased the liver TAS (by 10%) compared with control. The liver TAS in the CTB group was higher than in the Cd group (Fig. 4; $P < 0.01$). CTB supplementation to diets of quails caused a decline in liver TOS ($P < 0.01$) and OSI ($P < 0.01$) compared to the Cd group. The TAS and TOS levels for the control, CTB, and CTB+Cd groups

Table 2
Effect of CTB and/or Cd on laying performance of the quails.

Traits	Groups				SEM	*P*
	Control	CTB	Cd	CTB+Cd		
Egg weight (g)						
1 - 14 days	11.07	11.42	10.93	11.06	0.15	NS
15 - 28 days	11.39	11.62	10.85	11.25	0.20	NS
29 - 42 days	11.66^{a}	11.17^{ab}	10.85^{b}	10.99^{b}	0.12	**
43 - 56 days	11.44^{a}	11.08^{a}	10.44^{b}	11.00^{ab}	0.14	**
1 - 56 days	11.39^{a}	11.32^{a}	10.73^{b}	11.06^{ab}	0.09	**
Feed intake (g/quail/day)						
1 - 14 days	25.70	26.11	23.20	23.78	0.83	NS
15 - 28 days	25.50	26.72	23.93	23.71	1.37	NS
29 - 42 days	26.67^{a}	26.83^{a}	23.05^{b}	24.17^{b}	0.58	**
43 - 56 days	26.93^{a}	26.99^{a}	23.19^{b}	24.77^{ab}	0.81	*
1 - 56 days	26.20^{ab}	26.81^{a}	23.28^{c}	24.11^{bc}	0.59	**
Egg production % (egg production/quail/day)						
1 - 14 days	80.10^{a}	83.81^{a}	51.02^{b}	61.11^{b}	3.07	***
15 - 28 days	83.17^{a}	89.17^{a}	54.76^{b}	63.16^{b}	2.74	***
29 - 42 days	86.74^{a}	90.03^{a}	52.08^{b}	60.85^{b}	3.04	***
43 - 56 days	78.27^{a}	81.58^{a}	39.11^{c}	53.89^{b}	3.29	***
1 - 56 days	82.07^{a}	86.15^{a}	49.25^{c}	59.75^{b}	1.89	***
Feed conversion ratio (g feed intake / egg production x egg weight)						
1 - 14 days	2.92^{bc}	2.75^{c}	4.28^{a}	3.61^{ab}	0.19	***
15 - 28 days	2.71^{bc}	2.58^{c}	4.07^{a}	3.41^{ab}	0.19	***
29 - 42 days	2.68^{b}	2.67^{b}	4.21^{a}	3.73^{a}	0.18	***
43 - 56 days	3.11^{b}	3.06^{b}	5.92^{a}	4.26^{b}	0.34	***
1 - 56 days	2.81^{c}	2.75^{c}	4.45^{a}	3.67^{b}	0.11	***

CTB: Calcium tetraborate; Cd: Cadmium; SEM: Standart error of the mean. NS - non significant; NS: $P > 0.05$. * : $P < 0.05$; **: $P < 0.01$; *** : $P < 0.001$. a,b,c: Mean values with different superscripts within a row differ significantly.

Table 3
Effect of CTB and/or Cd on body weight, broken, soft-shelled, and extra-small eggs of the experimental groups.

Traits	Groups				SEM	*P*
	Control	CTB	Cd	CTB + Cd		
Initial BW (g)	209.00	209.87	209.35	209.53	4.89	NS
Final BW (g)	226.20^{a}	220.33^{ab}	205.80^{b}	208.60^{ab}	4.74	*
Broken egg (%)	0.0^{c}	0.07^{bc}	0.61^{a}	0.43^{ab}	0.08	**
Soft-shelled egg (%)	0.0^{b}	0.0^{b}	0.32^{a}	0.18^{a}	0.03	**
Extra-small egg (%)	0.04	0.0	0.04	0.04	0.03	NS

BW: Body weight; CTB: Calcium tetraborate; Cd: Cadmium; SEM: Standart error of the mean. NS - non significant; NS: $P > 0.05$. * : $P < 0.05$; **: $P < 0.01$; a,b,c: Mean values with different superscripts within a row differ significantly.

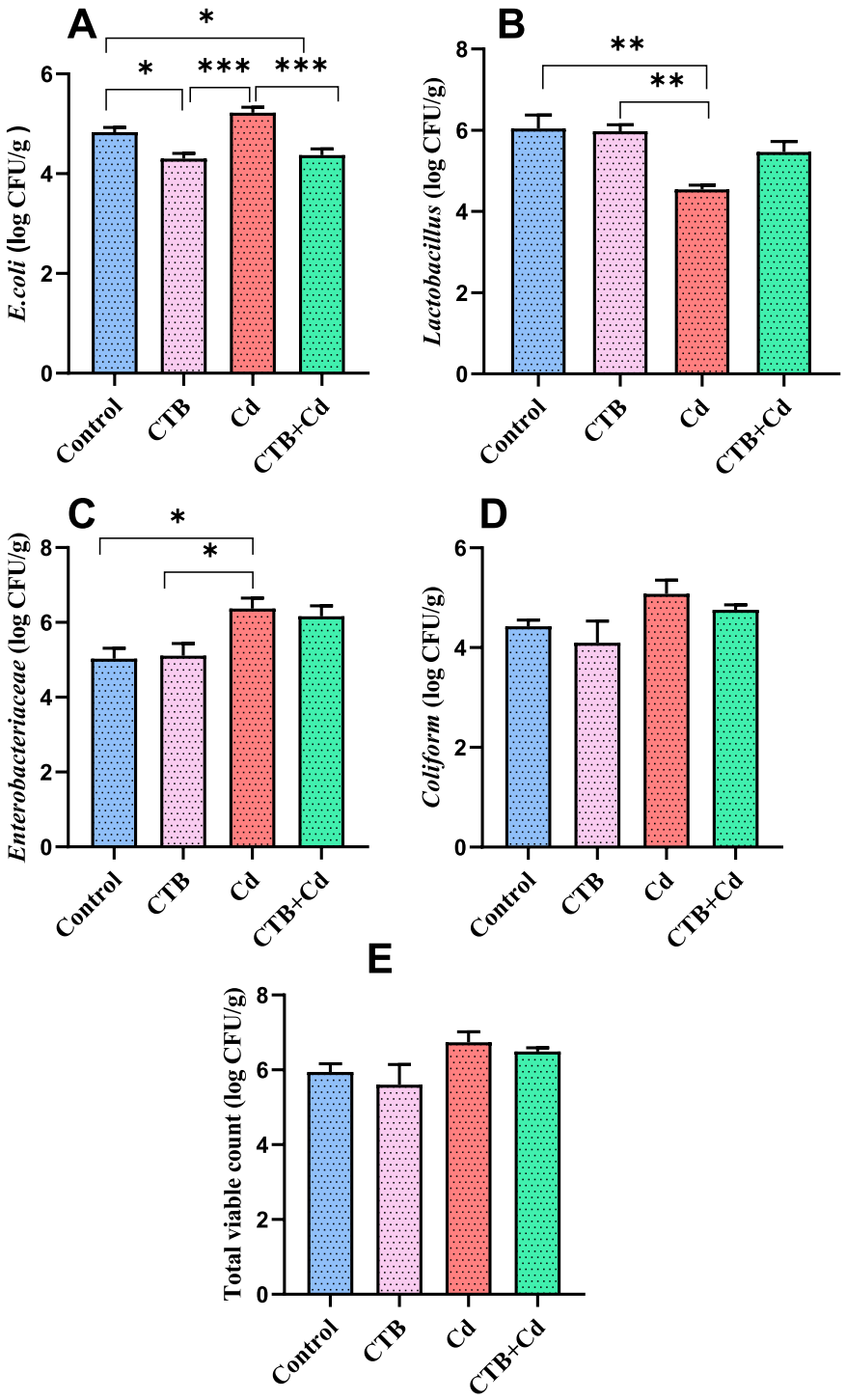

Fig. 3. Effect of CTB and/or Cd on cecal microflora $\log_{10}$ CFU g^{-1}. The bars represent the mean ± standard error of the mean. CTB: Calcium tetraborate; Cd: Cadmium; Statistical significance between groups is shown by:* : $P < 0.05$, **: $P < 0.01$, *** : $P < 0.001$.

were similar. While the lowest OSI value was determined in the CTB group, the highest OSI value was found in the Cd group ($P < 0.01$).

3.4. Histopathological assessment

Epicardial hemorrhages were noted in almost all animals supplementation Cd in the heart (Fig. 5A). This finding was not observed in the control and other treatment groups. In the liver of the animals in the Cd group, mild focal necrosis was found in the central and portal regions. In the kidneys, degeneration in the proximal tubules and glomeruli (Fig. 5B) and moderate interstitial bleeding and nephritis areas (Fig. 5C) in the distal regions were noted. Degenerated and necrotic seminiferous tubules were occasionally found in the testicles (Fig. 5D). In the treatment group, mild fatty degeneration was observed in the liver, while no necrosis was observed. While mild bleeding areas were observed in the kidneys, no degeneration or necrosis was observed. No findings were seen in the testicles either.

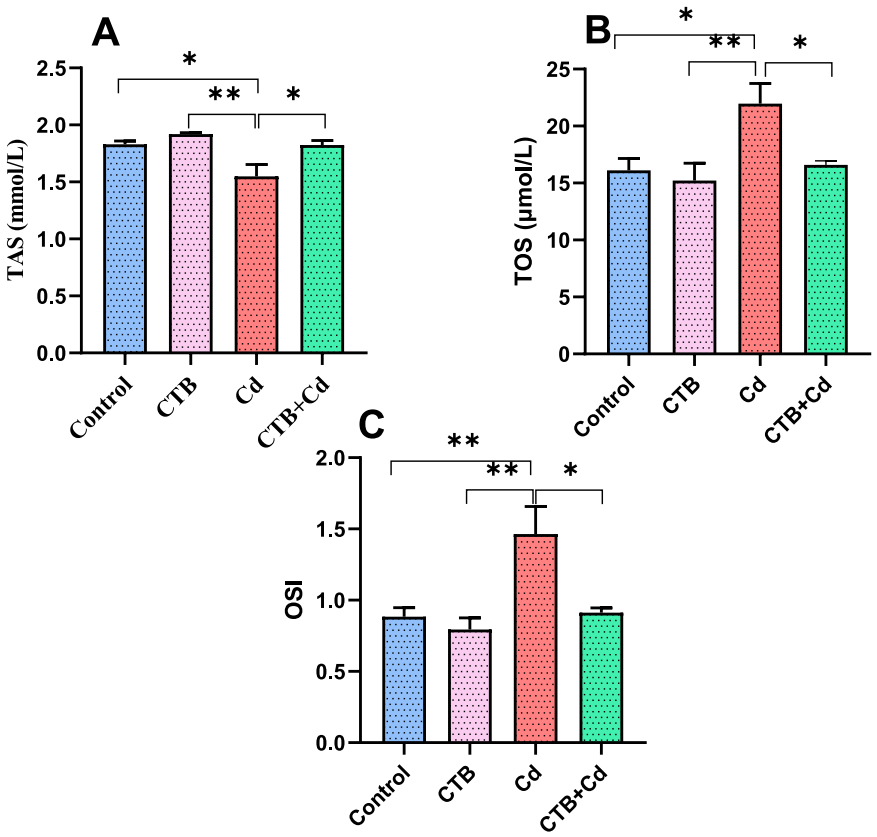

Fig. 4. The effect of CTB and/or Cd on TAS, TOS, and OSI in the liver. The bars represent the mean ± standard error of the mean (SEM). ANOVA and Tukey's post hoc test were used to compare the results among different treatment groups. CTB: Calcium tetraborate; Cd: Cadmium; TAS: Total antioxidative status; TOS: Total oxidative status; OSI: Oxidative stress index; Statistical significance between groups is shown by:* : $P < 0.05$, * *: $P < 0.01$.

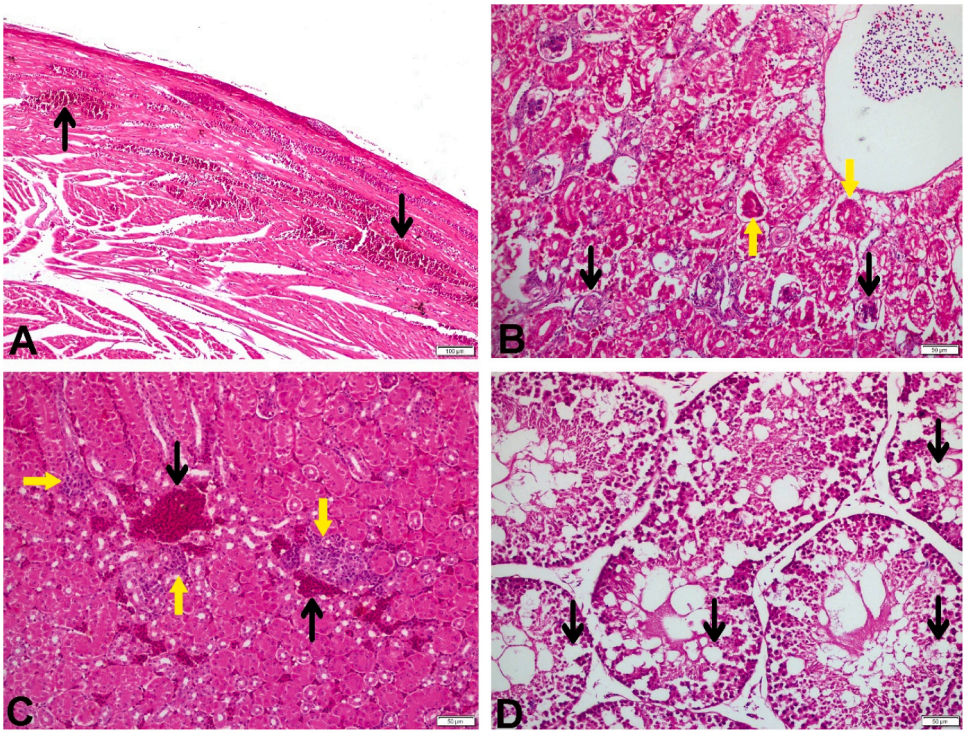

Fig. 5. A-D: Cadmium group. A. Epicardial hemorrhages in the heart (arrows). B. Degeneration in the proximal tubules (yellow arrows) and glomeruli (black arrows). C. Interstitial bleeding (black arrows) and nephritis areas (yellow arrows). D. Degenerated tubules in the testicles (arrows).

4. Discussion

Cd poses a risk to living things because it dissolves and decomposes easily (Dong et al., 2022). After Cd is ingested orally, it is excreted slowly through urine and feces; only a small part is absorbed. Cd is then retained in the liver, kidneys, and other target organs through the blood circulation system to exert its toxic effect (Yang et al., 2022). Cd, which is not essential for organisms and is common in the environment, causes liver damage and changes in histology and molecular biology (Bi et al., 2021; Elgharib et al., 2022). Cd binding to hepatic cell membranes induces oxidative stress, leads to oxidant stress in cells, and produces ROS by inhibiting the activity of hepatic antioxidant enzymes. Earlier studies have been conducted in various animals, including poultry, to mitigate Cd toxicity. These investigations using compounds with antioxidant properties and chelating agents to counteract Cd's harmful effects (Choong et al., 2014; Kar and Patra, 2021). Because Cd uses the same intestinal transporters as Ca in animals and humans, Ca content in the body can significantly affect Cd absorption in the gastrointestinal tract. Ca may regulate Cd-induced physiological or metabolic changes in organisms because it is chemically similar to Cd (Chen et al., 2021). Moreover, boric acid has attracted significant attention since it is an antioxidant (Coban et al., 2015) and can protect the liver (Ince et al., 2014). This study evaluated the effect of CTB on recovering the hazardous impact of Cd toxicity. .

In this study, 100 mg/kg Cd supplementation to diets of laying quails caused a statistically significant decrease in egg weight. Abou-Kassem et al. (2020) reported that Cd supplementation in laying quails significantly reduced egg weight during the experimental period. These results agreed with those obtained by Rahman (2007) and Olgun (2015). This may be attributed to Cd, an endocrine system disruptor that suppresses eggshell formation processes such as outer and inner shell membrane formation, Ca transport, and calcite crystal calcification in the egg formation pathway (Zhu et al., 2020). Our study showed that the supplementation of CTB to the Cd group improved egg weight but was not statistically significant. The present study recorded the lowest feed intake in the Cd and CTB+Cd groups. These findings are in line with Olgun (2015), who reported that adding 40 and 80 mg/kg Cd to the diet of laying quails for ten weeks caused a significant decrease in feed intake. Moreover, the results agree with the reports that adding 100 mg/kg Cd decreased feed intake (Al-Waeli et al. (2013). Hafez et al. (2023) stated that Cd (50 mg/kg diet) considerably reduced feed intake. Some studies on poultry have shown that dietary boron or boric acid in laying hens was no influence on body weight, feed intake, FCR, egg production, and egg weight (El-Saadany et al., 2017; Adarsh et al., 2021; Sizmaz et al., 2021). On the contrary, Bozkurt et al. (2012) reported increased feed intake after supplementation with 30 and 60 ppm boron. The current study demonstrated that egg production was decreased in the Cd group and CTB+Cd group. These findings are in line with Abou-Kassem et al. (2020), who determined that Cd decreased egg number in their study on quails. Similarly, Rahman (2007) reported that quails-fed diets supplemented with Cd exhibited significant decreases in egg production. Our results were also consistent with those of Olgun (2015). Rafieian-Naeini et al. (2021) demonstrated that Cd administration significantly decreased egg production. The significant reduction in egg number may be due to changes in the pathway of egg formation or suppression of Ca metabolism (Rahman, 2007). These results disagreed with those obtained by Toman et al. (2005), who found that using Cd showed no effect on the egg production of pheasants. These differences could be attributed to the differences in dose and exposure period of Cd. No significant difference was found among the groups fed by the diets-supplemented control group and the CTB group on egg production.

A study on quail reported that feed conversion became significantly worse with increasing Cd levels, similar to the present study's findings (Abou-Kassem et al., 2020). A study on broilers reported that Cd increased FCR (Al-Waeli et al., 2013). In the study conducted by Hafez et al. (2023), they observed that Cd (50 mg/kg diet) negatively impacted the FCR. Also, Rafieian-Naeini et al. (2023) found that FCR was adversely affected in Cd-challenged (subcutaneous injection of 10 mg Cd/kg BW) quail. Worsening feed efficiency upon exposure to Cd contamination may be due to reduced egg production. Long-term Cd

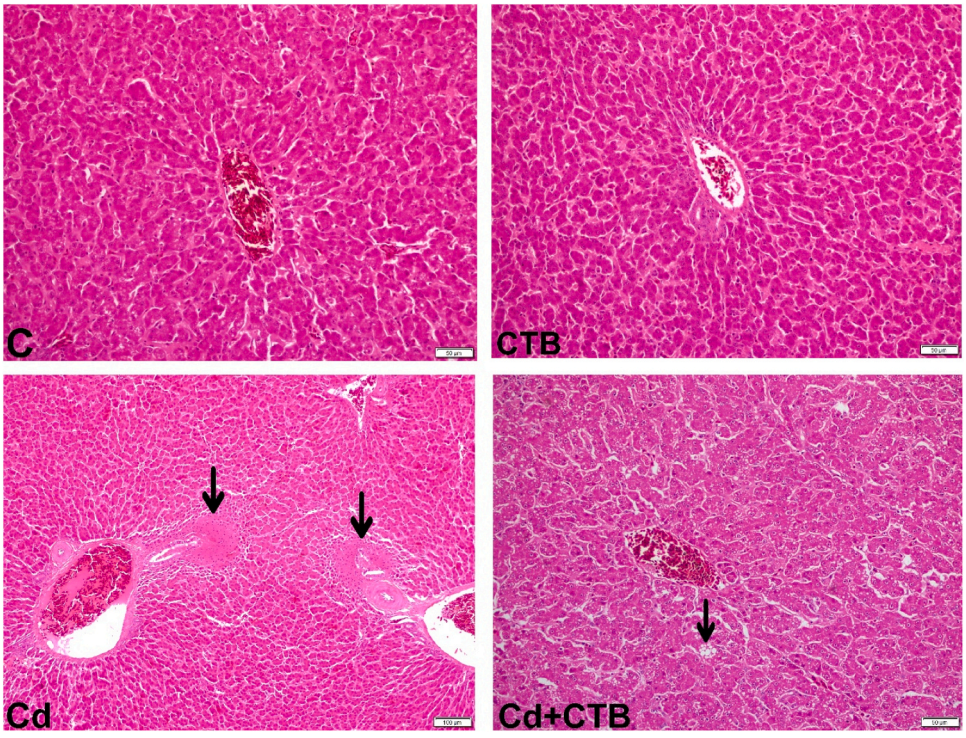

Fig. 6. Normal liver, C: control and CTB: Calcium tetraborate. Mild necrosis in the portal regions (arrows) in the cadmium (Cd) group. Mild fatty degeneration (arrow) in CTB+Cd.

exposure can reduce nutrient metabolism and feed utilization as it causes depletion of liver and muscle glycogen (Rahman, 2007). The findings of the present study demonstrated that CTB enhanced the FCR in the CTB+Cd group. Consistent with previous studies (Bokori et al., 1996), our results showed that the mortality rate showed no significant difference between the control group and the Cd group. This result disagrees with Abou-Kassem et al. (2020), who stated that Cd-polluted diets caused a 12.50% mortality rate. Moreover, Olgun (2015) also reported that a diet supplemented with 80 mg/kg Cd increased mortality in the quails. Similar to Rafieian-Naeini et al. (2021), found that Cd administration increased the mortality rate. In addition, the study also found that the Cd challenge has been reported to cause increased mortality in laying quail (Rafieian-Naeini et al., 2023).

This agreed with the findings of Abou-Kassem et al. (2020), who demonstrated that dietary Cd decreased body weight in Japanese quail layers. The findings of reducing body weight in Cd toxicity in the present experiment were almost similar to the results of Olgun (2015). Moreover, Hafez et al. (2023) revealed that supplementation of Cd (50 mg/kg diet) showed a significant decrease in the total body weight of quails. Likewise, Al-Waeli et al. (2013) reported that adding 100 mg/kg Cd caused significant adverse effects on body weight. However, Rafieian--Naeini et al. (2021) stated that Cd administration (1 mg/100 g BW at 10 and 11 wk of age) had no significant effect on body weight. Some studies on poultry have shown that supplemental boric acid improved performance (Bozkurt et al., 2012; Bozkurt and Kucukyilmaz, 2015). CTB + Cd group recorded higher body weight than the Cd group. However, dietary CTB supplementation had no significant effect on the body weight of quail. Higher dietary intakes of Ca may inhibit the absorption of dietary Cd from the gastrointestinal tract by directly competing with Cd for access to mineral transporters and downregulating these transporters' gene expression in the intestine. However, there needs to be more information on poultry (Kar and Patra, 2021).

Cd structurally resembles Ca and competitively binds to residues shared by Ca^{2+} while interacting with the intracellular estrogen receptor. This study showed that the supplementation of Cd to the diet significantly impacted broken and soft-shelled eggs. Olgun (2015) demonstrated that the dietary Cd supplementation negatively influenced the cracked-broken egg. Our study found that the proportion of broken and soft-shelled eggs in the Cd group was significantly higher than in the control and CTB groups. However, no significant difference was observed with the CTB+Cd group. In this study, the proportion of broken and soft-shelled eggs decreased after adding CTB because the dietary CTB supplementation can make more Ca^{2+} enriched in the eggshell (Xing et al., 2022). However, the differences in the proportion of broken and soft-shelled eggs among Cd and CTB+Cd groups were insignificant. Adarsh et al. (2021) reported that supplementing boron at a 40 ppm level increased eggshell thickness in laying hens.

Gut microbiota plays a crucial role in the gut health of poultry. The cecum harbors a more diverse, rich, and stable microbial community than any other intestinal segment. Cd, which directly affects the gut microbiota, can disrupt intestinal microbiota structure and composition, dysregulation in energy metabolism, disruption of the intestinal barrier, decrease expression and intracellular localization of adherens and tight junction proteins, and cause inflammatory response (Liu et al., 2014; Yang et al., 2022). This study examined the composition and function of cecal contents in quail for TVC, *Lactobacillus* spp., *Enterobacteriaceae*, coliforms, and *E. coli*. *E. coli*, *Lactobacillus* spp., and *Enterobacteriaceae* indicated significant group changes. Lactobacilli secrete anti-microbial substances like hydrogen peroxide, bacteriocins, and short chain fatty acids that either inhibit or kill pathogens, modulate the host's immune response to pathogens, prevent pathogen adherence, and compete with them for binding sites, all of which help to protect the intestinal barrier from infection (Dempsey and Corr, 2022). The *Lactobacillus* were enriched in the CTB+Cd group compared to the Cd group. *Enterobacteriaceae* and *E. coli* were proliferated induced by Cd. The number of *Lactobacillus* in the cecum was positively correlated with performance. The study has demonstrated that *Lactobacillus* spp. can produce bacteriocins and organic acids to inhibit pathogens (Chang et al., 2020). Compared with the control group, the *Lactobacillus* decreased, *Enterobacteriaceae* increased in the Cd group, and there was a significant difference in the Cd group. The coliforms increased in the Cd and CTB groups, but there was no significant difference. The TVC was higher in the Cd group than the control group, with no significant difference. The cecal microbiota between the control and CTB groups had no significant difference. This is consistent with previous studies showing that Cd exposure alters the microbiota composition (Liu et al., 2014; Yang et al., 2022). *Lactobacillus* can generate bacteriocins and organic acids, effectively suppressing pathogens and inflammation (Chang et al., 2020). Our results (Table 2) confirmed that CTB could improve the cecal microbiota.

Cd can deplete antioxidants in the body or weaken these defense systems. This can lead to a decrease in TAS levels. Low TAS levels indicate a reduced ability to protect cells against free radicals. With the entry of Cd into the body, the production of free radicals may increase, leading to elevated levels of oxidative stress. High TOS levels indicate increased free radicals in the body and the inadequacy of antioxidant defense systems to counter this increase. This leads to oxidative stress and negative consequences such as increased lipid peroxidation. The cellular-level mechanisms responsible for Cd's pro-oxidative effects are extensively reported (Brzoska and Rogalska, 2013; Khafaga et al., 2019; Dabrowski et al., 2020; Abdallah and El-Refaei, 2022; Elgharib et al., 2022). The findings of the study by Brzoska and Rogalska (2013) have suggested that the ability of Zn to prevent oxidative stress may be implicated in the mechanisms of its protective impact against Cd-induced bone damage. An increase in the values of TOS in the animals exposed to cadmium, compared to the CTB group, shows the extent of the intensity of this xenobiotic-induced oxidative stress. Therefore, the decreased TOS concentration of this toxic metal in liver tissue due to CTB supplementation may be one of the contributing factors to its protective effect and its antioxidative properties. Our findings are consistent with those of Ilhan et al. (2023). CTB partially prevented the Cd-caused increase in the OSI value and the TOS concentrations, showing that this compound prevents oxidative protein, lipid, and DNA damage in the liver tissue. While the protective mechanism of boric acid against oxidative damage remains incompletely understood, it is widely recognized for its role in cell membrane functions and enzymatic reactions (Cikler-Dulger and Sogut, 2020). Cikler-Dulger and Sogut (2020) reported that BA treatment significantly reduced ethanol-induced lipid oxidation and oxidative stress, as evidenced by the decrease in TOS and OSI. CTB, a boron compound, neutralizes free radicals in the body and reduces oxidative stress. This may explain the potential beneficial effects of boric acid in reducing cellular damage caused by Cd. The supplementation with CTB protected against this xenobiotic influence on the investigated parameters (Ilhan et al., 2023). In addition, this may be attributed to Ca's ability to protect organs from oxidative stress and protective effects on organ dysfunction (Chen et al., 2021).

It has been reported that Cd exposure induced the degradation of Purkinje cells in the chicken's cerebellum (Bi et al., 2021). In addition, Rafieian-Naeini et al. (2023) stated that histopathological alterations were found in Japanese quail under the Cd challenge. These results agree with the results obtained by Abdallah and El-Refaei (2022), who affirmed that the exposure to Cd was found to have caused severe histological changes. In this study, epicardial hemorrhages were found in the heart of Cd supplementation to diets of quails. However, this histopathological alteration was not observed in the control, CTB, and CTB+Cd groups. In this study, the CTB+Cd group attenuated Cd-caused pathological changes. Our histological examinations indicated that CTB ameliorated Cd-induced necrosis, degeneration, interstitial bleeding, nephritis, and necrotic seminiferous tubules. Ilhan et al. (2023) showed that boric acid ameliorated alcohol-induced degenerative changes in the kidney. In another study, Cikler-Dulger and Sogut (2020) reported decreased boric acid administration cell loss and leukocyte infiltration in

renal tubules. In a study conducted on mice, the protective role of Ca on organs was demonstrated by 100 g/kg Ca supplementation, partially attenuating pathological changes in the liver and kidneys (Chen et al., 2021). The CTB improved histomorphometric alterations induced by Cd. Boron compounds, especially boric acid and its salts, may have the capacity to bind heavy metals. This may reduce the circulation of Cd in the body and its spread to tissues. CTB may have a similar binding effect and, in this way, reduce the effects of Cd in the body. The beneficial effects of CTB can be attributed to its anti-inflammatory, antioxidant properties, and cytoprotective activities, which the boric acid it contains (Ince et al., 2014; Coban et al., 2015; Cikler-Dulger and Sogut, 2020; Ilhan et al., 2023).

5. Conclusion

As a result, according to productive performance, oxidative stress, cecal microflora, and histopathological findings, CTB, a boron compound, neutralizes free radicals in the body and reduces oxidative stress. This may explain the potential beneficial effects of boric acid in reducing cellular damage caused by Cd. Boric acid may act as a chelating agent that can bind heavy metals such as Cd. This means that boric acid can reduce the circulation and tissue accumulation of Cd in the body. CTB may have a similar binding effect and, in this way, reduce the effects of Cd in the body. More research is needed using different doses and animals on this compound's biological effects and general chelation therapy in Cd toxicity.

CRediT authorship contribution statement

Iflazoglu Mutlu Seda: Conceptualization, Formal analysis, Investigation, Supervision, Writing – review & editing. **Mutlu Muhsin:** Conceptualization, Formal analysis, Investigation, Project administration, Writing – original draft. **Simsek Ulku Gulcihan:** Conceptualization, Formal analysis, Methodology. **Iflazoglu Sera:** Formal analysis, Methodology. **Yilmaz Aysen:** Formal analysis, Methodology. **Karabulut Burak:** Formal analysis, Methodology. **Akdeniz Incili Canan:** Formal analysis, Methodology. **Cevik Aydın:** Formal analysis, Methodology. **Incili Gokhan Kursad:** Formal analysis, Methodology. **Tatli Seven Pinar:** Investigation, Writing – review & editing.

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.

Data Availability

Data will be made available on request.

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