https://doi.org/10.52973/rcfcv-e362883 Revista Científica, FCV-LUZ / Vol. XXXVI

Comparative evaluation of bio-protective effects of alive and dead (heat-inactivated) *Lactiplantibacillus plantarum* strains against Cadmium - induced toxicity in wistar rats

Evaluación comparativa de los efectos bioprotectores de cepas vivas y muertas (inactivadas por calor) de *Lactiplantibacillus plantarum* frente a la toxicidad inducida por Cadmio en ratas Wistar

***Tevhide Elif Güner*[1,*], *Hasan Susar*[2], *İhsan Kisadere*[3]**

[1] *Balıkesir University, Faculty of Veterinary Medicine, Department Food Hygiene and Technology. Balıkesir, Türkiye*
[2] *Balıkesir University, Faculty of Veterinary Medicine, Department of Pharmacology and Toxicology. Balıkesir, Türkiye*
[3] *Balıkesir University, Faculty of Veterinary Medicine, Department of Physiology.Balıkesir, Türkiye.*

**Corresponding author: elif.guner@balikesir.edu.tr*

ABSTRACT

This study was conducted to evaluate the bio - protective potential of alive and dead (heat - inactivated) *Lactiplantibacillus plantarum* strains against cadmium toxicity in Wistar rats. The aim was to determine whether alive and heat - inactivated *Lactiplantibacillus plantarum* strains could alleviate cadmium - induced inflammatory and oxidative stress responses and reduce serum cadmium levels in rats. The study included forty-eight male albino Wistar rats, which were distributed equally among six groups: control (n = 8), cadmium (n = 8), alive *Lactiplantibacillus plantarum* (n = 8), dead *Lactiplantibacillus plantarum* (n = 8), cadmium + alive *Lactiplantibacillus plantarum*, and cadmium + dead *Lactiplantibacillus plantarum*. Rats in the cadmium, cadmium + alive *Lactiplantibacillus plantarum*, and cadmium + dead *Lactiplantibacillus plantarum* groups received cadmium chloride (2 mg / kg) orally by gastric gavage three times a week for 4 weeks. Alive and dead *Lactiplantibacillus plantarum* (10^9 – 10^{10} cfu / mL) suspensions were administered to alive *Lactiplantibacillus plantarum*, dead *Lactiplantibacillus plantarum*, cadmium + alive *Lactiplantibacillus plantarum*, and cadmium + dead *Lactiplantibacillus plantarum* groups with the same frequency and duration. The control group received physiological saline. After experimental completion, decapitation was performed, and serum samples were collected for cadmium concentrations, cytokines (TNF - α, IL - 6, IL - 10, IL - 1β), and antioxidant (Malondialdehyde, superoxide dismutase, glutathione peroxidase, catalase) levels. cadmium administration caused a significant increase in serum TNF - α and IL - 6 levels but decreased IL - 1β levels ($P < 0.05$). Superoxide dismutase and glutathione peroxidase levels were reduced, but Malondialdehyde concentrations elevated in only the cadmium - exposed group ($P < 0.05$). *Lactiplantibacillus plantarum* administrations alleviated the inflammatory response by providing a significant decrease in IL - 6 levels, especially in the heat - inactivated form (cadmium + dead *Lactiplantibacillus plantarum*) ($P < 0.05$). Both alive and dead forms showed partial recovery trends in superoxide dismutase, glutathione peroxidase, and catalase. *Lactiplantibacillus plantarum* exhibited partially anti-inflammatory, antioxidant, and metal-binding properties against cadmium toxicity in both forms and presented a usable bioprotective model in terms of food safety and veterinary toxicology when applied with a milk matrix.

Key words: Cadmium, inflammation, L. plantarum, oxidative stress, paraprobiotic

RESUMEN

Este estudio se llevó a cabo para evaluar el potencial bioprotector de cepas vivas y muertas (inactivadas por calor) de *Lactiplantibacillus plantarum* frente a la toxicidad por cadmio en ratas Wistar. El objetivo fue determinar si las cepas vivas y termoinactivadas de *Lactiplantibacillus plantarum* podían atenuar las respuestas inflamatorias y de estrés oxidativo inducidas por cadmio y reducir los niveles séricos de cadmio en las ratas. Cuarenta y ocho ratas macho albinas Wistar se dividieron en seis grupos iguales : control (n = 8), cadmio (n = 8), *Lactiplantibacillus plantarum* viva (n = 8), *Lactiplantibacillus plantarum* muerta (n = 8), cadmio + *Lactiplantibacillus plantarum* viva y cadmio + *Lactiplantibacillus plantarum* muerta. Las ratas de los grupos cadmio, cadmio + *Lactiplantibacillus plantarum* viva y cadmio + *Lactiplantibacillus plantarum* muerta recibieron cloruro de cadmio (2 mg / kg) por vía oral mediante gavage gástrico, tres veces por semana durante 4 semanas. Las suspensiones de *Lactiplantibacillus plantarum* viva y muerta (10^9 – 10^{10} UFC / mL) se administraron a los grupos *Lactiplantibacillus plantarum* viva, *Lactiplantibacillus plantarum* muerta, cadmio + *Lactiplantibacillus plantarum* viva y cadmio + *Lactiplantibacillus plantarum* muerta con la misma frecuencia y duración. El grupo control recibió solución salina fisiológica. Posteriormente, los animales fueron decapitados y se recolectaron muestras de suero para la determinación de las concentraciones séricas de cadmio, citocinas (TNF - α, IL - 6, IL - 10, IL - 1β) y niveles de antioxidantes (malondialdehído, superóxido dismutasa, glutation peroxidasa, catalasa). La administración de cadmio provocó un aumento significativo de los niveles séricos de TNF - α e IL - 6, pero una disminución de los niveles de IL - 1β ($P < 0,05$). Los niveles de superóxido dismutasa y glutation peroxidasa se redujeron, mientras que las concentraciones de malondialdehído se elevaron únicamente en el grupo expuesto a cadmio ($P < 0,05$). Las administraciones de *Lactiplantibacillus plantarum* atenuaron la respuesta inflamatoria al provocar una disminución significativa de los niveles de IL - 6, especialmente en su forma inactivada por calor (cadmio + *Lactiplantibacillus plantarum* muerta) ($P < 0,05$). Tanto las formas vivas como las muertas mostraron tendencias de recuperación parcial en los niveles de superóxido dismutasa, glutation peroxidasa y catalasa. *Lactiplantibacillus plantarum* presentó propiedades parcialmente antiinflamatorias, antioxidantes y de unión a metales frente a la toxicidad por cadmio en ambas formas, y mostró un modelo bioprotector utilizable en términos de seguridad alimentaria y toxicología veterinaria cuando se aplicó junto con una matriz láctea.

Palabras clave: Cadmio, inflamación, L. plantarum, estrés oxidativo, paraprobiótico

Received: 23/12/2025 Accepted: 23/02/2026 Published: 28/03/2026

INTRODUCTION

Cadmium (Cd) is an environmentally prevalent metal that exerts pronounced toxic effects on biological systems even at relatively low exposure levels. It enters the ecosystem through industrial processes, phosphate fertilizers, plastic manufacturing, batteries, and wastewater. Due to its lengthy biological half - life, Cd accumulates particularly in the liver, kidneys, lungs, brain, and cardiovascular system and leads to deterious effects [1 , 2]

Environmental or food - borne exposure to Cd results in disruption of the redox balance, oxidative stress, and triggering of the inflammatory response in the organism. Cd ions bind to sulfhydryl groups and inhibit the action of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). This disturbance promotes oxidative stress, manifested by increased lipid peroxidation (LPO), genomic damage, and intracellular accumulation of reactive oxygen species (ROS) [3 , 4].

In addition to oxidative stress, Cd is also known to increase the synthesis of proinflammatory cytokines such as tumor necrosis factor alpha (TNF - α), interleukin - 6 (IL - 6), and IL - 1beta (β) by activating the nuclear factor kappa – light – chain - enhancer of activated B cells (NF - κB) signaling pathway. These mechanisms trigger systemic inflammation, leading to disruption of tissue integrity and loss of metabolic homeostasis [5 , 6].

Transmission of Cd to humans and animals through the food chain is one of the most significant aspects of exposure. It accumulates in soil and water, then enters the food chain through plant products, feeds, and therefore animal foods. In terms of food safety and environmental toxicology, this condition presents a major public health concern [7]. For this reason, interest in biological detox techniques that will reduce the bioavailability of Cd and facilitate its elimination from the body is increasing day by day (d). Certain probiotic bacteria, particularly *Lactiplantibacillus plantarum* (Lp), have been shown in recent studies to have a protective effect against heavy metal poisoning [8 , 9].

It has been suggested that the protective effect of Lp is multifaceted. The cell surface carboxyl and phosphate groups of Lp bind Cd^{2+} ions and restrict the metal's absorption from the gastrointestinal system by biosorption [10 , 11]. Furthermore, the production of short - chain fatty acids and antioxidant metabolites supports epithelial barrier integrity by lowering intestinal pH and reduces the transfer of toxic ions into the circulation. These mechanisms suggest that Lp functions as an antioxidant and immunomodulatory bioprotective agent not only at the local intestinal level but also at the systemic level [12 , 13].

Lactiplantibacillus plantarum is a probiotic microorganism that is commonly found in fermented products of plant origin and can also adapt to a milk environment and is therefore frequently used in functional food formulations. Probiotics play a crucial role in bridging the gap between veterinary toxicology and food safety by maintaining their viability in biological environments such as milk and enhancing their bioavailability against toxic chemicals [14 , 15].

In previous research, no comprehensive study was found to comparatively evaluate the live and heat-inactivated (paraprobiotic) forms of Lp under heavy metal toxicity conditions and to reveal their bioprotective mechanisms at the cellular level in a holistic manner. Additionally, Zhai *et al.* [16] investigated the short-term protective effects of heat-inactivated and live Lp CCFM8610 against acute Cd poisoning; however, the study had limited parameters and a 48 - hour experimental duration.

The present study aimed to overcome this limitation and evaluate the effects of alive and inactive forms of Lp on some serum antioxidant and inflammatory (cytokine) parameters in a holistic manner under longer - term Cd exposure. Thus, probiotics' paraprobiotic potential, which does not depend on viability, is being examined from a fresh angle in the context of toxicity and food safety. The study's novel experimental model fills a major gap in the literature. The information gathered is expected to offer a scientific foundation for lowering the risks of foodborne illness and creating probiotic - based bioprotective products.

MATERIAL AND METHODS

Ethics statement

This research was conducted with the approval of the Balıkesir University Animal Experimentation Local Ethics Committee (Ethics Committee Approval No : 2024 / 11 - 10). All experimental procedures were conducted in accordance with the "European Council Directive 2010 / 63 / EU on the Welfare and Experimental Use of Animals" and the "Balıkesir University Experimental Animal Ethics Committee Working Procedures and Principles." Throughout the experiment, unnecessary stress was avoided in the animals, the number of animals used was minimized, and the 3R (Replacement, Reduction, Refinement) principles were observed.

Animal material

In this study, 48 three – week - old male Wistar albino rats (*Rattus norvegecis*) with an average body weight (Kern, EW 620-3NM, Germany) of 200 ± 30 g were used. Male Wistar rats were supplied by the Experimental Animal Production, Care, Application, and Research Center of Balıkesir University. Following acclimatization, the animals were allocated into six groups of equal size using a randomization procedure. Throughout the experimental period, rats were maintained in plastic cages under controlled environmental conditions with a 12 hours (h) light/12 h dark cycle. The room temperature and a relative humidity were 23 ± 2 °C, 50 ± 10 %, respectively. Standard rat chow and fresh water were provided to the animals *ad libitum* during the study.

Experimental groups

Cadmium Group (Cd) **:** Cadmium chloride ($CdCl_2$) was administered by oral gavage at a dose of 2 mg/kg, three times per week for four weeks [17].

Alive Lp Group (ALP) **:** Suspension containing approximately $10^9 – 10^{10}$ cfu / mL of live bacteria was given by oral gavage three times per week for four weeks [18].

Cd + ALP Group **:** $CdCl_2$ (2 mg / kg) and $10^9 – 10^{10}$ cfu / mL live Lp suspension were administered by oral gavage three times per week for four weeks [17 , 18].

Dead Lp Group (DLP) **:** Approximately $10^9 – 10^{10}$ cfu / mL of heat - inactivated bacterial suspension was applied (by oral gavage) three times a week for four weeks [18].

Cd + DLP Group : $CdCl_2$ (2 mg / kg) and $10^9 – 10^{10}$ cfu / mL of inactivated Lp suspension were administered orally (by gavage) three times per week for four weeks [17 , 18].

Control Group (C) : Physiological saline was administered orally via gavage for the same periods of time as stated above.

Analysis of cadmium - resistant strain

Four Lp strains were screened for Cd tolerance by assessing minimum inhibitory and lethal concentration thresholds (MIC and MLC), and the strain exhibiting the highest resistance was chosen for subsequent experiments. Each strain was grown in de Man, Rogosa, and Sharpe (MRS) broth containing different Cd concentrations with some adaptations to the method of Zhai *et al*. [19].

In this study, Cd concentrations ranging from 0.1 to 200 mg / L were used, and all required concentrations were prepared by making appropriate dilutions from a 1.000 mg / L $CdCl_2$ stock solution. Lp strains inoculated into each tube were incubated (Memmert, model E412.0361, Schwabach, Germany) under microaerophilic conditions at 37 °C for 24 – 48 hours (h). Bacterial growth was monitored by measuring the optical density (OD_{600}) at 600 nm and by a visual assessment of turbidity (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany). MIC was determined as the lowest concentration of cadmium that inhibited observable bacterial growth, while MLC was identified as the lowest concentration resulting in the absence of growth after transfer to fresh, Cd-free MRS agar plates. Each experiment was independently repeated three times.

At the end of the incubation period, the Lp Biofen (DSMZ 16627) strain, which was able to grow at the highest Cd concentration, was selected as the most Cd - resistant strain for use in the rest of the study.

Preparation of bacterial suspensions

The selected Lp strain was cultivated in 10 mL of de MRS broth (Merck, Darmstadt, Germany) and incubated at 30 °C for 18 – 20 h in a temperature-controlled incubator (Memmert, model E412.0361, Schwabach, Germany). At the end of the incubation, the cultures were centrifuged (Universal 320 R, Hettich Zentrifugen, Germany) at 4200 × G for 5 minutes (min) in a centrifuge cooled to 4 °C. After removing the supernatants, the bacterial pellets were resuspended in 10 mL of sterile skim milk powder solution (Merck, 115363, Darmstadt, Germany). The final bacterial suspension was prepared to contain approximately $10^9 – 10^{10}$ cfu/mL, as described previously with minor modifications [8 , 18].

Inactivation process of Lp

Bacterial suspensions were heat-inactivated by autoclaving (Hirayama, Hiclave HV85, Japan) at 121 °C for 15 min, following previously described procedures for paraprobiotic preparations [14 , 20]. Loss of viability was confirmed by the absence of growth after incubation on MRS agar.

Detecting of serum cadmium concentrations

The wet ash digestion process was used to mineralize whole blood samples at atmospheric pressure. Each sample's 1 mL of blood was combined with 5 mL of concentrated nitric acid (HNO_3) and 2 mL of hydrogen peroxide (H_2O_2), then heated to 90 °C. Until a definitive solution was found, the procedure was repeated. After, the solutions were put into volumetric flasks and filled with distilled water to a volume of 10 mL. Prior to analysis, prepared samples were kept at 4 °C in a refrigerator (Siemens, model KD56NNW22N, Germany). Cd concentrations were determined using inductively coupled plasma–optical emission spectrometry (ICP-OES; Optima 7300, PerkinElmer, USA) following the method of Saglam *et al*. [21].

Detection of serum Malondialdehyde concentrations and some antioxidant levels

Serum samples were analyzed using commercially obtained Enzyme-Linked Immunosorbent Assay (ELISA) kits, following the protocols provided by the manufacturer, and measurements were performed with an ELISA microplate reader (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany), the concentrations of Malondialdehyde (MDA) (BT Lab, E0156Ra, China), GPx values (BT Lab, E1172Ra, China), SOD (BT Lab, E1444Ra, China), and CAT (BT Lab, E0869Ra, China) enzyme activities were determined [8].

Detection of some serum cytokine levels

Serum TNF - α [(Bioassay Technology Laboratory (BT Lab), E0764Ra, China)], IL – 1 - β [(BT Lab, E0119Ra, China)], IL - 6 [(BT Lab, E0135Ra, China)], and IL - 10 [(BT Lab, E0108Ra, China)] were determined by ELISA employing commercial kits, and absorbance readings were obtained using an ELISA reader (SPECTROstar Nano, BMG LABTECH GmbH, Ortenberg, Germany) [8].

Experimental workflow

The overall experimental workflow, including Cd exposure, probiotic administration, sample collection and analysis steps, is illustrated in FIG 1.

Statistical analysis

Statistical analyses were carried out using the Statistical Package for the Social Sciences software (SPSS version 25.0; SPSS Inc., Chicago, IL, USA). Mean ± standard error (SEM) is used to express the results. The Shapiro - Wilk test was used to evaluate data distribution and homogeneity of variance. The Duncan multiple comparison test and one-way analysis of variance were used to investigate group differences. Statistically significant differences were defined as $P < 0.05$.

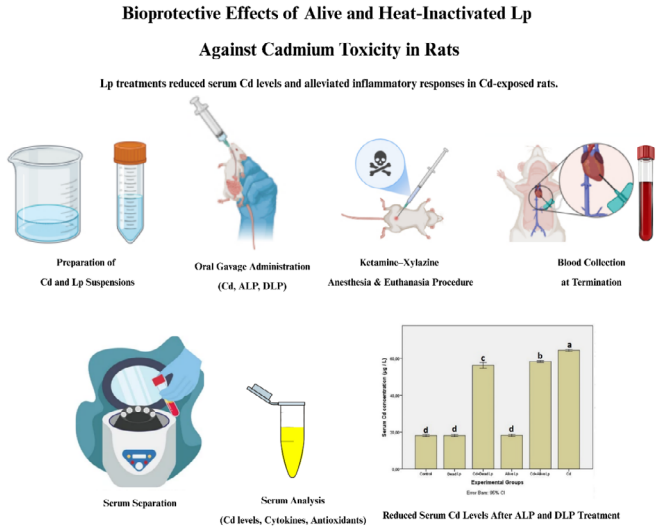

FIGURE 1. Experimental design and overview of the study. Cadmium (Cd) and Lactiplantibacillus plantarum (Lp) suspensions were prepared and administered to rats by oral gavage for 4 weeks. Experimental groups received Cd alone or Cd in combination with alive (ALP) or dead (DLP) L. plantarum. At the end of the experimental period, animals were anesthetized, blood samples were collected, serum was separated, and Cd concentrations, antioxidant parameters, and cytokine levels were analyzed. The bar graph illustrates changes in serum Cd concentrations following ALP and DLP treatments. Values are expressed as mean ± SEM. Different letters (a - d)above bars indicate statistically significant differences among groups ($P < 0.05$)

RESULTS AND DISCUSSION

Serum cadmium concentrations

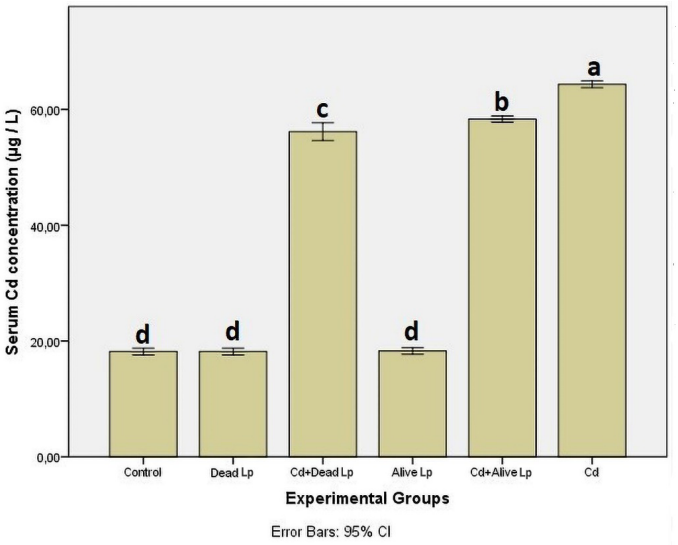

FIGURE 2. Serum cadmium (Cd) concentrations (µg/L) in the experimental groups after the 4-week treatment period. Control animals received no treatment. Dead Lp represents rats administered heat-inactivated Lactiplantibacillus plantarum, while Alive Lp indicates rats receiving viable L. plantarum. Cd + Dead Lp and Cd + Alive Lp groups received cadmium in combination with dead or alive L. plantarum, respectively. The Cd group received cadmium alone. Values are presented as mean ± 95 % confidence interval (CI). Different letters (a–d) above bars indicate statistically significant differences among groups ($P < 0.05$)

Serum Cd concentration was found to be higher in the Cd group than in other experimental groups in the present study ($P < 0.05$) (FIG. 2). Besides, its concentration was detected lower in the Cd + ALP and Cd + DLP (especially) groups compared to the Cd group in this study ($P < 0.05$). In addition, it was not determined any change among the groups (C, ALP, and DLP) regarding serum Cd levels ($P > 0.05$), which were likely increased only due to fress drinking water and free dietary intake. It was reported that chronic Cd exposure led to an increase in blood Cd concentrations and altered some antioxidant and cytokine levels in living beings [8]. In this study, Cd administration increased the serum Cd concentrations in the Cd group when compared to the C group. These findings were similar to previous studies [8 , 22 , 23]. On the other hand, the Lp - administrated groups (Cd + ALP and Cd + DLP) had lower serum Cd concentrations than the Cd group in this investigation. The systemic bioavailability of Cd was considerably decreased by both treatments, while the death strain's (DLP) decline was somewhat more than the alive strain's (ALP).

This result is consistent with studies showing that Lp inhibits metal absorption by binding to Cd^{2+} ions in the intestinal environment, thus reducing serum levels and systemic bioavailability via the gut-liver axis [8 , 9 , 16 , 24 , 25 , 26]. Moreover, following heat treatment, the bacterial cell wall's enhanced exposure of functional groups, including phosphate, carboxyl, and teichoic acids, may have expanded the number of binding sites for Cd ions.

Some of these locations may have been masked by surface proteins and metabolic processes in living bacteria. As a result, the ion exchange sites on the surface of dead Lp would be more accessible compared to the living form, and their biosorption capability might have risen [10].

Serum malondialdehyde concentrations and some antioxidant levels

Cadmium treatment resulted in increased serum MDA concentrations relative to the C group ($P < 0.05$) (TABLE I). Alive or dead (heat - inactivated) Lp administrations could not ameliorate the MDA concentrations in the Cd + ALP and Cd + DLP groups when compared to the Cd group ($P > 0.05$). On the other hand, the serum MDA levels of the Cd + ALP and Cd + DLP (especially) groups partially decreased following Lp administration; however, this decline was not statistically significant ($P > 0.05$).

Compared with the control group, the Cd group exhibited significantly lower serum GPx levels ($p < 0.05$). Administration of alive or dead (heat - inactivated) Lp administrations did not affect the serum GPx levels in the Cd + ALP and Cd + DLP groups compared to the Cd group ($P > 0.05$). All administrations (ALP, DLP, and Cd) had no effect on serum CAT enzyme activities, although all of the experimental groups' SOD enzyme activities were lower than those of the C group ($P < 0.05$).

On the other hand, the alive Lp strain administration showed a tendency toward higher SOD and GPx activity in the Cd + ALP group compared to the Cd group. In comparison to the active strain (ALP), the mean GPx level seemed to be marginally greater in the dead strain administered group (Cd + DLP). In addition, the heat - inactivated (dead) strain showed a tendency toward higher CAT activity compared to the Cd group ($P > 0.05$).

TABLE I
Malondialdehyde concentrations and some antioxidant levels in the experimental groups (mean ± SEM)

Groups	n	MDA (mmol / mL)	GPx (U / mL)	SOD (ng / mL)	CAT (ng / mL)
Control	8	0.68 ± 0.09^{b}	1042.50 ± 27.17^{a}	22.56 ± 3.37^{a}	5.21 ± 0.89 n.s
ALP	8	0.95 ± 0.16^{b}	940.62 ± 4.27^{ab}	12.65 ± 1.77^{b}	4.14 ± 1.29 n.s
DLP	8	0.81 ± 0.17^{b}	988.12 ± 90.03^{ab}	14.19 ± 4.43^{b}	5.10 ± 0.85 n.s
Cd	8	1.80 ± 0.27^{a}	892.50 ± 40.91^{b}	6.55 ± 2.45^{b}	2.98 ± 0.24 n.s
Cd + ALP	8	1.60 ± 0.07^{a}	897.50 ± 15.08^{b}	6.64 ± 1.79^{b}	3.35 ± 0.31 n.s
Cd + DLP	8	1.48 ± 0.02^{a}	906.25 ± 10.72^{b}	6.76 ± 0.43^{b}	4.08 ± 0.37 n.s

MDA: Malondialdehyde; GPx: Glutathione peroxidase; SOD: Superoxide dismutase; CAT: Catalase; ALP: Alive Lactiplantibacillus plantarum; DLP: Dead Lactiplantibacillus plantarum; Cd: Cadmium; Cd + Alive Lp: Cadmium and Alive Lactiplantibacillus plantarum; CD + DLP: Cadmium and Dead Lactiplantibacillus plantarum. n: number of animals in each experimental group; mmol: milimol; mL: mililiter; U: Unit; ng: nanogram; The data is represented as the mean ± standard error; n.s: nonsignificant. *Different letters (a – c) in the same column indicate a statistically significant difference between groups (P < 0.05).

Previously, many studies have demonstrated that Cd stimulates the formation of ROS and disrupts intracellular redox balance in living organisms [1 , 4]. In the present study, Cd administration increased the serum MDA (the principal product of polyunsaturated fatty acid peroxidation) concentrations in the Cd group compared to the C group.

The present results are in agreement with earlier reports [27 , 28 , 29]. In contrast, administration of Lp was associated with a partial reduction in serum MDA concentrations in the Cd + ALP and particularly the Cd + DLP groups; however, this decrease did not reach statistical significance in the current study. These findings were compatible with previous research [8 , 9]. Conversely, it was reported that neither Cd nor the other experimental groups (*Bacillus coagulans*, *L. plantarum*, and inulin) showed any discernible variations in MDA concentrations throughout the course of 42 d in rats [30].

This discrepancy is thought to be due to significant differences in the experimental conditions of the two studies. The significantly lower Cd dose (200 µg / rat / d) used by Jafarpour *et al*. [30] compared to the dose used in the present experiment, the administration of probiotics in synbiotic form (*L. plantarum* + inulin), the different Lp strains used, and the longer timeframe (21 and 42 d) for assessing oxidative stress parameters may have led to the different observed MDA responses.

Because these variables can directly affect the severity of oxidative stress, the observation that Cd administration significantly increased MDA levels, while this increase was partially limited in the Lp - treated groups, although not statistically significant, can be explained by these methodological differences.

It has been reported that the ability of defense enzymes (such as SOD, GPx, and CAT) to function is limited when Cd ions interact with cellular sulfhydryl groups and bind to the active sites of antioxidant enzymes [31 , 32].

In the present study, exposure to cadmium resulted in reduced of GPx levels in the Cd group compared to the C group. Although serum CAT enzyme activities were not affected, serum SOD levels were reduced by Cd administration in the Cd group compared to the C group in this study.

Xue *et al*. [33] reported a reduction in serum CAT and GPx levels following cadmium exposure ($CdCl_2$, 6 mg / kg, b.w.), whereas SOD activity in serum remained unchanged in Sprague Dawley female rats. Conversely, it has been shown that cadmium exposure at a dose of 2.04 mg/mL administered orally for 28 days had no measurable effect on serum GSH concentrations or SOD and CAT activities in female rats [8]. These differences may have varied depending on different doses or gender.

On the other hand, the alive Lp strain administration showed a nonsignificant tendency toward higher SOD and GPx activity in the Cd + ALP group compared to the Cd group. In comparison to the active strain (ALP), the mean SOD and GPx level seemed to be marginally greater (not meaningful) in the dead strain administered group (Cd + DLP).

In addition, the heat-inactivated (dead) strain showed a tendency toward higher CAT activity compared to the Cd group. This phenomenon could be due to functional groups on the cell surface still being able to bind Cd ions even after the cell is no longer viable. However, there was no discernible difference among the groups regarding serum CAT levels in rats.

These results were in line with research in the literature that showed heat-inactivated Lp's ability to bind Cd and how it regulated oxidative stress [16]. The administration of Lp decreased the oxidative load brought on by exposure to Cd in both situations. This partial recovery may be indicative of Cd accumulation within the tissues, which keeps the pressure on antioxidant enzyme systems partially intact. In addition, these results demonstrate that Lp exerts a protective effect against Cd toxicity through both physicochemical (adsorption) and biological (activation of antioxidant enzymes) mechanisms. Likewise, numerous probiotic strains have been documented in the literature to mitigate oxidative stress resulting from heavy metal exposure and maintain antioxidant enzyme activities [9 , 34].

Some serum cytokine levels

In this investigation, serum TNF - α levels were shown to be greater in the Cd group than in the C group (P < 0.05) (TABLE II). Besides, IL - 6 levels were observed to be higher in the Cd group than in the C group (P < 0.05), although serum IL - 10 levels were unaffected by any administration (P > 0.05).

TABLE II
Some serum cytokine levels in the different experimental groups (mean ± SEM)

Groups	*n*	TNF - α (ng / L)	IL - 6 (ng / L)	IL - 10 (pg / mL)	IL - 1β (pg / mL)
Control	8	2.25 ± 0.05^{c}	0.85 ± 0.01^{c}	158.41 ± 52.31 n.s	32.96 ± 9.45^{a}
ALP	8	2.32 ± 0.16^{bc}	1.08 ± 0.06^{bc}	117.56 ± 15.47 n.s	14.97 ± 2.54^{b}
DLP	8	2.18 ± 0.16^{c}	1.19 ± 0.10^{bc}	96.46 ± 11.25 n.s	19.91 ± 8.60^{ab}
Cd	8	2.69 ± 0.11^{a}	1.76 ± 0.30^{a}	82.98 ± 2.10 n.s	3.28 ± 0.59^{b}
Cd + ALP	8	2.63 ± 0.11^{ab}	1.34 ± 0.11^{ab}	89.79 ± 8.84 n.s	8.98 ± 1.27^{b}
Cd + DLP	8	2.42 ± 0.07^{ab}	1.14 ± 0.13^{bc}	88.17 ± 12.42 n.s	4.23 ± 2.08^{b}

TNF – α: Tumor necrosis factor alpha; IL – 6: Interleukin - 6; IL – 10: Interleukin – 10 ; IL - 1β: Interleukin - 1 beta ; ALP: Alive Lactiplantibacillus plantarum; DLP: Dead Lactiplantibacillus plantarum; Cd: Cadmium; Cd + Alive Lp: Cadmium and Alive Lactiplantibacillus plantarum; CD + DLP: Cadmium and Dead Lactiplantibacillus plantarum. n: number of animals in each experimental group; ng : nanogram; L: Liter; pg: pictogram; mL: mililiter; The data is represented as the mean ± standard error; n.s: nonsignificant. *Different letters (a – c) in the same column indicate a statistically significant difference between groups (P < 0.05)

An important decrease in IL - 6 levels was observed only in the heat-inactivated strain (Cd + DLP) (P < 0.05), while there was a decreasing trend in the alive strain (Cd + ALP) (P > 0.05) in the present study. Serum IL - 1ß levels were detected to be lower in the Cd, ALP, Cd + ALP, and also Cd + DLP groups compared to the C group (P < 0.05), interestingly. However, serum IL-1β levels in the Cd + ALP group showed a partial restoration when compared with the Cd-only group. Cadmium is a potent pro - oxidant and inflammatory agent due to its tendency to accumulate in living tissues, long half - life, and capacity to disrupt redox balance. Prior research indicates that Cd - induced oxidative stress activates the NF - κB complex and promotes the transcription of proinflammatory mediators such as TNF - α, IL - 6, IL - 1β, and cyclooxygenase - 2 [5 , 6]. Consistent with this mechanism, Cd exposure was associated with higher serum levels of TNF-α and IL-6, whereas IL-10 concentrations showed a non-significant downward trend.

According to Choudhury *et al*. [35], the immune response switches to a proinflammatory axis when IL - 10 levels fall, preventing the resolution of inflammation. On the other hand, it was reported that Cd administration did not cause any significant changes regarding serum TNF - α and IL - 1β levels in female rats [8].

In a previous study, it was also suggested that Cd administered orally at a dose of 2 mg/kg over a 4-week period failed to significantly affect serum IL-1β, but led to increased TNF-α levels in male Wistar rats [36]. In agreement with previous findings, similar results were reported by Han *et al*. [37] in mice.

Additionally, a decline in serum IL-1β levels was observed following Cd administration. According to previous studies, the production of this cytokine is suppressed at high or prolonged exposure and increased at low levels [36 , 38]. This could be explained by Cd blocking the caspase - 1 enzyme, which stops IL - 1β from maturing. Lp administration resulted in a partial improvement in cytokine profiles in the Cd + ALP and Cd + DLP groups compared to the Cd group.

An important decrease in IL - 6 levels was observed only in the heat-inactivated strain (Cd + DLP), while a decreasing trend was observed in the alive strain (Cd + ALP). Furthermore, no significant difference was found among the groups (Cd, Cd + ALP, and Cd + DLP) regarding TNF - α levels. However, a partial recovery was observed in the Cd + ALP group's serum IL - 1β levels compared to the Cd group. Despite Cd exposure, the administration of alive Lp resulted in a nonsignificant partial decrease in TNF - α and IL - 6 levels, a nonsignificantly improvement in IL - 1β, and a not meaningful recovery in IL - 10.

These findings demonstrate that Lp has a limited immunomodulatory capacity, and it can regulate cytokine balance. Similarly, it has been reported in the literature that this probiotic suppresses the Cd - induced inflammatory response and improves the cytokine profile [8 , 39 , 40]. The attenuation of the systemic inflammatory response associated with Cd toxicity after Lp administration suggests that this bacterium both directly interacts with immune cells and creates an indirect regulatory effect through the intestinal barrier [9 , 16].

CONCLUSIONS

When serum Cd levels and antioxidant enzyme activities of rats were evaluated, it was determined that DLP administration was more effective than ALP administration in effectively reducing serum Cd levels (as a metal binding agent), thereby a partially decreasing lipid peroxidation and oxidative stress. Moreover, it was also observed that DLP administration had a slightly better effect (nonsignificant) on serum pro-inflammatory cytokine activity, while ALP had a partialy positive effect (nonsignificant) on anti-inflammatory cytokine activity.

These findings suggest that different forms of probiotic bacteria (live or heat - inactivated) may be effective as bioprotective agents in reducing the bioavailability of foodborne contaminants and controlling oxidative stress and inflammation.

Furthermore, Lp, naturally occurring in plant - based fermented foods and adaptable to milk matrices, is thought to contribute to the development of innovative approaches for food safety and veterinary public health.

In this context, probiotic - containing dairy products can be considered functional foods that help reduce systemic oxidative damage and inflammation, particularly due to toxic metals such as Cd. The applicability of Lp in food matrices offers a practical advantage for use in field conditions.

Furthermore, the ability of paraprobiotic forms to exhibit similar protective effects without requiring viability significantly contributes to product stability and safety. This allows the use of both live and inactivated probiotics in functional dairy products.

Funding

The financial support for this study was supplied by the Scientific Research Unit of Balıkesir University (BAP Project No : 2024 / 031).

Conflict of Interest

The authors of this article declare that there are no potential conflicts of interest.

BIBLIOGRAPHIC REFERENCES

[1] Arroyo VS, Flores KM, Ortiz LB, Gómez-Quiroz LE, Gutiérrez-Ruiz MC. Liver and cadmium toxicity. J. Drug Metab. Toxicol. [Internet]. 2012; S5:001. doi: https://doi.org/qt7c

[2] Prozialeck WC, Edwards JR, Woods JM. The vascular endothelium as a target of cadmium toxicity. Life Sci. [Internet]. 2006; 79(16):1493–1506. doi: https://doi.org/dsj2nn

[3] El-Sharaky AS, Newairy AA, Badreldeen MM, Eweda SM, Sheweita SA. Protective role of selenium against renal toxicity induced by cadmium in rats. Toxicol. [Internet]. 2007; 235(3):185–193. doi: https://doi.org/cvq9c8

[4] Nemmiche S, Chabane-Sari D, Kadri M, Guiraud P. Cadmium chloride-induced oxidative stress and DNA damage in the human Jurkat T cell line is not linked to intracellular trace elements depletion. Toxicol. In Vitro. [Internet]. 2011; 25(1):191–198. doi: https://doi.org/b8ckj8

[5] Chen X, Bi M, Yang J, Cai J, Zhang H, Zhu Y, Zheng Y, Liu Q, Shi G, Zhang Z. Cadmium exposure triggers oxidative stress, necroptosis, Th1/Th2 imbalance and promotes inflammation through the TNF-α/NF-κB pathway in swine small intestine. J. Hazard. Mater. [Internet]. 2022; 421:126704. doi: https://doi.org/gzq3tf

[6] Zhang Y, Li Y, Zhang J, Qi X, Cui Y, Yin K, Lin H. Cadmium induced inflammation and apoptosis of porcine epididymis via activating RAF1/MEK/ERK and NF-κB pathways. Toxicol. Appl. Pharmacol. [Internet]. 2021; 415:115449. doi: https://doi.org/gjr3xk

[7] Gök-Metin Z, Yavuz CI. Çevresel ve mesleksel kadmiyum etkilenimi ve sağlık etkileri. Sagl. Toplum [Internet]. 2022 [cited 12 Nov 2025]; 32(2):26–37. Available in: https://goo.su/7qTx0

[8] Kisadere I, Guner B, Tavsanli H, Aydin MF, Usta M. Influence of *Lactobacillus plantarum* administration on some serum/tissue (uterus) antioxidant and cytokine levels in female rats exposed to cadmium (Cd). Turk. J. Vet. Anim. Sci. [Internet]. 2023; 47(3):270–280. doi: https://doi.org/qpgp

[9] Zhai Q, Wang G, Zhao J, Liu X, Narbad A, Chen YQ, Zhang H, Tian F, Chen W. Protective effects of *Lactobacillus plantarum* CCFM8610 against chronic cadmium toxicity in mice indicate routes of protection besides intestinal sequestration. Appl. Environ. Microbiol. [Internet]. 2014; 80(13):4063–4071. doi: https://doi.org/f56mzh

[10] Teemu H, Seppo S, Jussi M, Raija T, Kalle L. Reversible surface binding of cadmium and lead by lactic acid and bifidobacteria. Int. J. Food Microbiol. [Internet]. 2008; 125(2):170–175. doi: https://doi.org/fp86tp

[11] Zhai Q, Tian F, Wang G, Zhao J, Liu X, Cross K, Zhang H, Narbad A, Chen W. The cadmium binding characteristics of a lactic acid bacterium in aqueous solutions and its application for removal of cadmium from fruit and vegetable juices. RSC Adv. [Internet]. 2016; 6(8):5990–5998. doi: https://doi.org/gfzgx3

[12] Karczewski J, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJM, Wells JM. Regulation of human epithelial tight junction proteins by *Lactobacillus plantarum in vivo* and protective effects on the epithelial barrier. Am. J. Physiol. Gastrointest. Liver Physiol. [Internet]. 2010; 298(6):G851–G859. doi: https://doi.org/cgb7ss

[13] Wang W, Xu C, Zhou X, Zhang L, Gu L, Liu Z, Ma J, Hou J, Jiang Z. *Lactobacillus plantarum* combined with galactooligosaccharides supplement: a neuroprotective regimen against neurodegeneration and memory impairment by regulating short-chain fatty acids and the c-Jun N-terminal kinase signaling pathway in mice. J. Agric. Food Chem. [Internet]. 2022; 70(28):8619–8630. doi: https://doi.org/qt7d

[14] Guner TE, Akyol BA, Tavsanli H, Gokbulut C. Effects of different inactivation and activation treatments applied to lactic acid bacteria on AFM1 detoxification in milk. Food Biosci. [Internet]. 2025; 69:106974. doi: https://doi.org/qt7f

[15] Ma X. Heavy metals remediation through lactic acid bacteria: Current status and future prospects. Sci. Total Environ. [Internet]. 2024; 946:174455. doi: https://doi.org/g94znd

[16] Zhai Q, Wang G, Zhao J, Liu X, Tian F, Zhang H, Chen W. Protective effects of *Lactobacillus plantarum* CCFM8610 against acute cadmium toxicity in mice. Appl. Environ. Microbiol. [Internet]. 2013; 79(5):1508–1515. doi: https://doi.org/f4k39c

[17] Kisadere I, Aydin MF, Usta M, Donmez N. Protective effects of oral melatonin against cadmium induced neurotoxicity in Wistar rats. Arh. Hig. Rada Toksikol. [Internet]. 2021 [cited 12 Nov 2025]; 72(2):157–163. Available in: https://goo.su/FYWr

[18] Banwo K, Alonge Z, Sanni AI. Binding capacities and antioxidant activities of *Lactobacillus plantarum* and *Pichia kudriavzevii* against cadmium and lead toxicities. Biol. Trace Elem. Res. [Internet]. 2021; 199(2):779–791. doi: https://doi.org/ghfmp8

[19] Zhai Q, Xiao Y, Zhao J, Tian F, Zhang H, Narbad A, Chen W. Identification of key proteins and pathways in cadmium tolerance of *Lactobacillus plantarum* strains by proteomic analysis. Sci. Rep. [Internet]. 2017; 7:1182. doi: https://doi.org/f97ch5

[20] Hashemi SMB, Amiri MJ. Detoxification of aflatoxin M1 in sarshir by viable and nonviable *Limosilactobacillus reuteri* and *Limosilactobacillus rhamnosus*: Kinetic, equilibrium and desorption studies. Int. Dairy J. [Internet]. 2022; 127:105223. doi: https://doi.org/grkrwm

[21] Saglam HS, Altundag H, Atik YT, Dundar MS, Adsan O. Trace elements levels in the serum, urine, and semen of patients with infertility. Turkish. J. Med. Sci. [Internet]. 2015; 45(2): 443–448. doi: https://doi.org/qt7g

[22] Andjelkovic M, Buha-Djordjevic A, Antonijevic E, Antonijevic B, Stanic M, Kotur-Stevuljevic J, Spasojevic-Kalimanovska V, Jovanovic M, Boricic N, Wallace D, Bulat Z. Toxic effect of acute cadmium and lead exposure in rat blood, liver, and kidney. Int. J. Environ. Res. Public Health. [Internet]. 2019; 16(2):274. doi: https://doi.org/qpgs

[23] Nasiadek M, Danilewicz M, Klimczak M, Stragierowicz J, Kilanowicz A. Subchronic exposure to cadmium causes persistent changes in the reproductive system in female Wistar rats. Oxid. Med. Cell. Longev. [Internet]. 2019; 2019:6490820. doi: https://doi.org/g8sh2q

[24] Zhai Q, Tian F, Zhao J, Zhang H, Narbad A, Chen W. Oral administration of probiotics inhibits absorption of the heavy metal cadmium by protecting the intestinal barrier. Appl. Environ. Microbiol. [Internet]. 2016; 82(14):4429–4440. doi: https://doi.org/qt7h

[25] Zhai Q, Liu Y, Wang C, Zhao J, Zhang H, Tian F, Lee YK, Chen W. Increased cadmium excretion due to oral administration of *Lactobacillus plantarum* strains by regulating enterohepatic circulation in mice. J. Agric. Food Chem. [Internet]. 2019; 67(14):3956–3965. doi: https://doi.org/qt7j

[26] Zhu J, Yu L, Shen X, Tian F, Zhao J, Zhang H, Chen W, Zhai Q. Protective effects of *Lactobacillus plantarum* CCFM8610 against acute toxicity caused by different food-derived forms of cadmium in mice. Int. J. Mol. Sci. [Internet]. 2021; 22(20):11045. doi: https://doi.org/gpn654

[27] Abd-Allah MM, Ramadan AA, Said NM, Ibrahim IH, Abdel-karim EA. Effects of cadmium chloride and glyphosate on antioxidants as biochemical biomarkers in Nile tilapia. J. Aquac. Res. Dev. [Internet]. 2019; 10(1):1000561. doi: https://doi.org/qt7k

[28] Kara H, Karatas F, Canatan H. Effect of single dose cadmium chloride administration on oxidative stress in male and female rats. Turk. J. Vet. Anim. Sci. [Internet]. 2005 [cited 13 Aug 2025]; 29(1):37–42. Available in: https://goo.su/GY2Rw

[29] Zhang Y, Li Z, Kholodkevich S, Sharov A, Feng Y, Ren N, Sun K. Cadmium-induced oxidative stress, histopathology, and transcriptome changes in the hepatopancreas of freshwater crayfish (*Procambarus clarkii*). Sci. Total Environ. [Internet]. 2019; 666:944–955. doi: https://doi.org/gv5gg6

[30] Jafarpour D, Shekarforoush SS, Ghaisari HR, Nazifi S, Sajedianfard J, Eskandari MH. Protective effects of synbiotic diets of *Bacillus coagulans*, *Lactobacillus plantarum* and inulin against acute cadmium toxicity in rats. BMC Complement. Altern. Med. [Internet]. 2017; 17(1):291. doi: https://doi.org/gbg8bm

[31] Cuypers A, Plusquin M, Remans T, Jozefczak M, Keunen E, Gielen H, Opdenakker K, Nair AR, Munters E, Artois TJ, Nawrot T, Vangronsveld J, Smeets K. Cadmium stress: an oxidative challenge. Biometals. [Internet]. 2010; 23(5):927–940. doi: https://doi.org/ddcb5p

[32] Genchi G, Sinicropi MS, Lauria G, Carocci A, Catalano A. The effects of cadmium toxicity. Int. J. Environ. Res. Public Health [Internet]. 2020; 17(11):3782. doi: https://doi.org/gpwrpb

[33] Xue Y, Huang F, Tang R, Fan Q, Zhang B, Xu Z, Sun X, Ruan Z. Chlorogenic acid attenuates cadmium-induced intestinal injury in Sprague–Dawley rats. Food Chem. Toxicol. [Internet]. 2019; 133:110751. doi: https://doi.org/qt7p

[34] Majlesi M, Gheisari HR, Shekarforoush SS, Abhari K, Dahesh S, Pashangeh S. Protection of probiotic *Bacillus coagulans* and *Lactobacillus plantarum* against cadmium toxicity in rats. Comp. Clin. Pathol. [Internet]. 2023; 32:789–797. doi: https://doi.org/qt7q

[35] Choudhury C, Mazumder R, Biswas R, Sengupta M. Cadmium exposure induces inflammation through the canonical NF-κB pathway in monocytes/macrophages of *Channa punctatus* Bloch. Fish Shellfish Immunol. [Internet]. 2021; 110:116–126. doi: https://doi.org/gjr3xs

[36] Kisadere I. The effects of melatonin treatment on some serum immunoregulatory cytokine levels in rats exposed to chronic cadmium toxicity. Kocatepe Vet. J. [Internet]. 2020 [cited 12 Aug 2025]; 13(3):262–266. Available in: https://goo.su/Aioqw

[37] Han P, Tian X, Wang H, Ju Y, Sheng M, Wang Y, Cheng D. Purslane (*Portulaca oleracea* L.) polysaccharide relieves cadmium-induced colonic impairments by restricting Cd accumulation and inhibiting inflammatory responses. Int. J. Biol. Macromol. [Internet]. 2024; 257(1):128500. doi: https://doi.org/g5dx3p

[38] Napolitano J, Liu M, Bao S, Knoell D. Cadmium suppresses IL-1β production and release in monocytes (P3010). J. Immunol. [Internet]. 2013; 190(1):114.6. doi: https://doi.org/qt7s

[39] Kizir D, Toraman E, Karaman M. Molecular changes associated with inflammation and reproduction in cadmium-induced testicular toxicity: Mitigating effect of *Lactobacillus plantarum*. Biol. Trace Elem. Res. [Internet]. 2025; 203:5768–5775. doi: https://doi.org/qt7t

[40] Zhang Q, Shang X, Geng L, Che X, Wei H, Tang S, Xu W. Dietary selenium-rich *Lactobacillus plantarum* alleviates cadmium-induced oxidative stress and inflammation in bulatmai barbel *Luciobarbus capito*. Fishes. [Internet]. 2023; 8(3):136. doi: https://doi.org/qt7v