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BATTLING LEAD TOXICITY: QUERCETIN'S PROTECTIVE MECHANISMS IN LAYING QUAILS

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Abstract
Lead (Pb) is a pervasive environmental pollutant, prevalent in industrial areas, posing significant threats to both human and animal health. Exposure to Pb occurs through various avenues including industrial pollution, agricultural processing, and contaminated soil and feed. This cumulative toxin extensively accumulates in vital organs such as the brain, liver, kidney, bone, and hemopoietic system, instigating a spectrum of biochemical, physiological, behavioral, and morphological disorders. Central to the mechanism of Pb toxicity is oxidative damage. In response to this challenge, natural compounds with dual chelating and antioxidant properties have been explored for their potential in mitigating the adverse effects of Pb exposure.

Keywords: Lead toxicity, environmental pollutant, chelating agents, antioxidant compounds, oxidative damage.

Introduction

Lead (Pb) is one of the important environmental pollutants that is widely found in industrial areas and has toxic effects on humans and animals (Mustika 2017). The general environment as well as industrial pollution, food/agricultural processing, soil, and feed contamination cause Pb exposure (Farag et al. 2018). Pb is a cumulative toxin that prevents growth by accumulating in various organs such as brain, liver, kidney, bone and hemopoietic system, and causes biochemical, physiological, behavioral and morphological disorders. Oxidative damage plays a role in the entire mechanism of Pb. (Ebrahimi et al. 2015; Farag et al. 2018). Natural compounds with both chelating and antioxidant activities are used to reduce the negative effects of Pb toxicity (Singh et al. 2018).

Quercetin is a powerful antioxidant flavonoid that is found in high amounts in fruits and vegetables.

Quercetin is mostly found in onions, grapes, strawberries, cherries, broccoli, and citrus fruits (Anand David et al. 2016). Quercetin has been stated to be a powerful antioxidant, thanks to its ability to scavenge free radicals. Ortho phenolic groups of quercetin B ring chelate by binding Pb (Singh et al. 2018). Quercetin can treat diseases caused by oxidative damage by increasing the body's total antioxidant activity and related antioxidant enzymes (Xu et al. 2019). Studies have shown that quercetin is a versatile antioxidant with protective abilities against tissue damage. In addition to its antioxidant effect, quercetin also has antibacterial and anti-inflammatory effects (Anand David et al. 2016). As a result of these effects, it has been reported to improve physical performance and inhibit lipid peroxidation (Wang et al. 2018). In a study, it was stated that in laying hens fed with 0.367-0.369 g/kg quercetin for 8 weeks, quercetin increased the performance by modulating the intestinal environment and quercetin has a functional feed additive potential in animal production (Liu et al.

2014). We hypothesize that dietary quercetin may play a role in ameliorating Pb-induced toxicity. For this purpose, we studied the effect of quercetin on performance, survivability, egg quality, cecal microflora population, and nutrient digestibility against Pb induced toxicity in laying quails.

1. 0. Material and Methods

A total of 112 laying Japanese quails (5 weeks old, *Coturnix coturnix Japonica*) were obtained from a commercial quail producer company. This study was conducted with the ethics committee approval of the Veterinary Control Institute Animals Experiments Ethics Committee (dated 05.29.2018, decision no: 2018/01). The quails were kept at a temperature of 22±2 ^{0}C and 16L:8D hours/daylighting period. The quails were allocated to 4 experimental groups, each containing 28 quails with 4 repetitions. The quails were fed 1 of 4 diets for 8 weeks: 1- Control, 2- Pb (100 mg/kg Pb), 3- Quercetin (400 mg/kg Quercetin), 4- Pb+Quercetin (100 mg/kg Pb +400 mg/kg Quercetin), The quercetin was supplied by a commercial firm (PureBulk Inc Roseburg, OR, USA, Quercetin dihydrate pure) and Pb in the form of lead acetate was supplied by Merck (Blei(II)-AcetateTrihydrateReinst). Quails were fed with a diet including 17% crude protein and 2800 kcal/kg metabolizable energy (Table 1) and water ad libitum. The nutrient composition of the diet used in the study was determined (Crampton and Maynard 1938; Carpenter and Clegg 1956; NRC 1994; AOAC 2000). The experiment lasted 8 weeks. The performance parameters were evaluated during the period of the experiment. Feed intake (g/bird/day), egg production (egg production/bird/day) and feed conversion ratio (g feed intake/egg production x egg weight) were calculated. The individual body weight of quails was determined at the beginning and end of the experiment. Mortality was recorded daily during the period of the experiment. A total of 288 eggs (4 groupx4 replicantx6 eggsx 3 times) were collected 6 eggs from each subgroup in the 6th, 7th, and 8th weeks of the study for egg quality parameters. After the collected eggs were kept at room temperature for 24 hours, the shell weight, shell thickness (IP54, Qinghai, China), yolk index, albumen index, and Haugh unit (Haugh 1937) values of the eggs were examined.

Table1: Ingredients and nutrient composition of experimental diet (%)[a]

Ingredients	%
Maize	66.27
Soybean meal (44% CP)	24.44
Wheat bran	1.31
Salt	0.25
L-Lysine hydrochloride	0.21
L-Treonine	0.13
Sodium bicarbonate	0.10
DL-Methionine	0.12
Vitamin-Mineral premix[b]	0.32
Limestone	5.46
Calcium phosphate	1.39
Total	100

Nutritional composition	
Dry matter, %	89.90
Crude protein, %	17.00
Crude cellulose, %	3.31
Ether extract, %	1.89
Crude ash, %	9.78
Calcium[c]	2.50
Phosphorus[c]	0.35
Sodium[c]	0.16
Lysine[c]	1.00
Threonine[c]	0.75
ME, kcal/kg[c]	2800

[a]Quercetin(400 mg quercetin per kg diet) was added to the basal diet. [b]Vitamin-mineral premix (per 1kg): 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. [c]Calculated.

At the last week of the experiment, 6 animals from each group were individually caged to observe nutrient digestibility. The lignin indicator method was used. The excreta samples were daily collected from each cage over a seven-day collection period. The diet and excreta samples were oven-dried (60 °C for 36-48 h), grounded, and analyzed (Van Soest 1991; AOAC 2000). The protein content of excreta samples was corrected based on uric acid using the following equation (Rotter et al. 1989). The nutrient digestibility was calculated following equation. 100-[100 x (%ADL in diet / %ADL in feces) x (%nutrient in feces / %nutrient in diet)]

6 quails from each group which close to the average body weight of the groups were randomly selected and slaughtered by decapitation. Approximately 1 g of cecal digesta samples were aseptically collected and diluted with 9 mL of 0.1% peptone water until they were diluted to $10^{-7}$and homogenized for 1 min to prepare for serial dilutions. *Lactobacillus* spp. were counted on Man, Rogosa and Sharpe agar (Merck, Darmstadt, Germany), while coliforms were counted on Violet Red Bile Agar (Merck, Darmstadt, Germany). Bacterial numbers were reported as $\log_{10}$ CFU per gram of cecal contents (ISO 1998; ISO 2006).

SPSS (IBM 2012) was used for all statistical analyses. One-way analysis of variance (ANOVA) was used to compare group means followed by Duncan's post hoc test. The results were considered significant at $P<0.05$.

3. 0. Results and Discussion

One of the most widespread in the environmental toxins is lead (Pb) which induces oxidative stress via the generation of reactive oxygen species. Pb-induced oxidative stress negatively affects feed conversion ratio and growth performance in poultry (Ebrahimi et al. 2015). Phytoantioxidants have been reported to be effective in alleviating toxic effects of Pb. Quercetin has an antioxidant and metal chelating property. Thus, the properties of quercetin have made it very promising as a therapeutic in the field of heavy metal toxicity (Flora et al. 2012).In the present study, Pb and quercetin did not significantly affect body weight (Table 2, P>0.05). However, this result contradicts that Ibitoye et al. (2011) observed that supplemental dietary Pb decreased the body weight in broilers.

Table 2: Body weight of quails

	Control	Pb	Quercetin	Pb +Quercetin	P
Initial BW (g)	184.26±4.39	184.79±2.92	184.17±3.62	184.30±3.91	NS
Final BW (g)	238.62±4.13	233.96±3.06	237.12±3.98	235.64±4.96	NS

Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin, BW: Body weight.
The data presents as mean and standard error. NS: non significant; NS: P>0.05.

In this study, feed intake was observed the highest in quails fed the only quercetin (400 mg/kg), while the lowest feed intake was recorded for the only Pb group (Fig. 1A). Studies in poultry, it has been reported that fed by a diet supplemented Pb reduces feed intake (Taha et al. 2019; Seven et al. 2012). These findings are in line with Alagawany et al. (2018), who determined that Pb decreased feed intake in their study on quails. Similarly, Ebrahimi et al. (2015) reported that 200 mg/kg lead acetate supplemented broilers exhibited significant decreases in feed intake. However, Ibitoye et al. (2011) found that Pb (200 mg/kg) did not affect feed intake in broiler. Alagawany et al. (2018) showed that supplementation of Pb caused a statistically significant decrease in egg production in layer quails. However, they stated that compared to the control, other performance parameters such as egg weight and egg mass were not affected by Pb toxicity. Pb did not affect feed conversion ratio (P>0.05). These results agreed with those obtained by Taha et al. (2019). Ibitoye et al. (2011) reported no effects of 200 mg/kg Pb in broiler diets on feed conversion ratio. On the contrary, Ebrahimi et al. (2015) reported a significant decrease in feed conversion ratio. The differences in performance could be attributed to the differences in dose, form, or exposure period of Pb. Moreover, this may explain by the adverse effects of Pb on health, taste, digestion, and absorption (Iflazoglu Mutlu et al. 2021).

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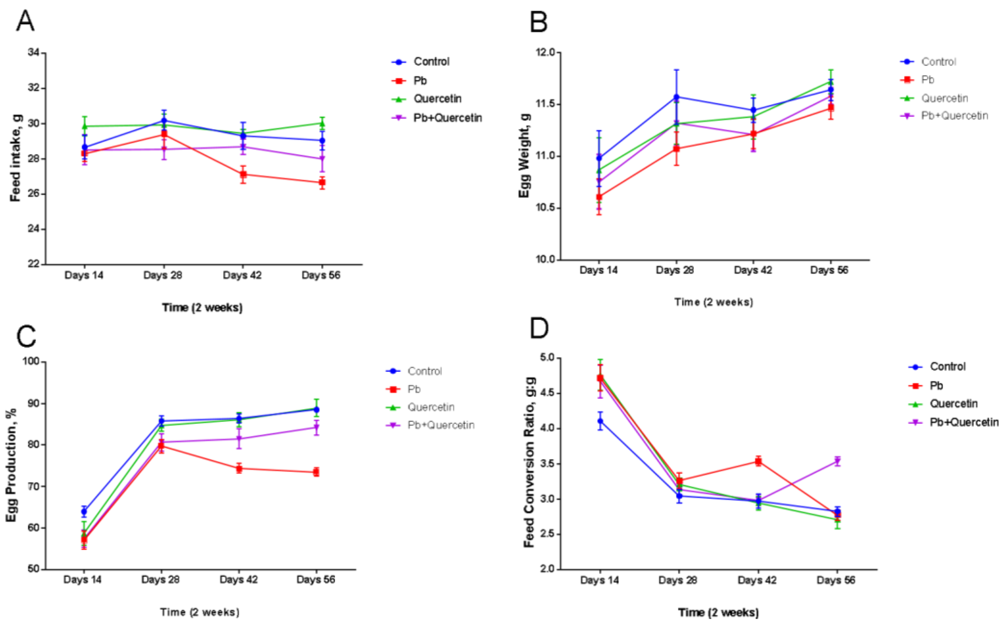
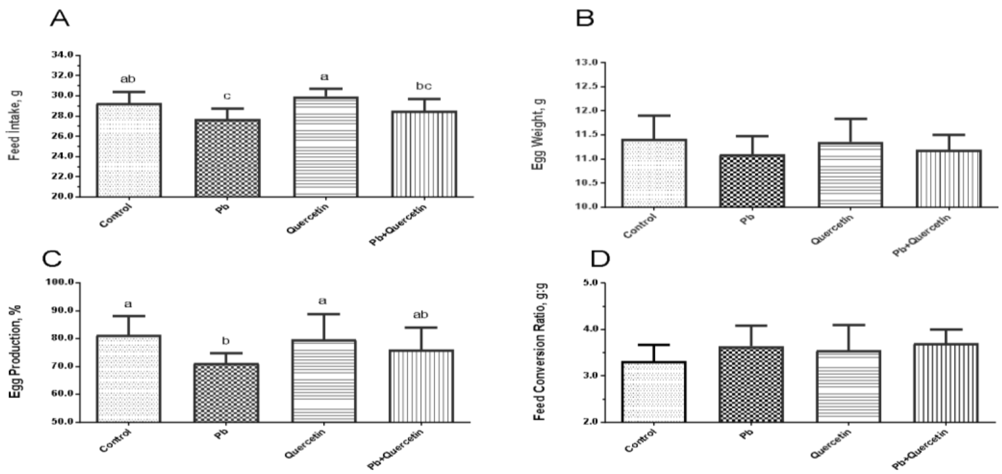


The graphics represent the standard error of the mean. Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin. **Fig. 1: The effects of quercetin on feed intake (Panel A), egg weight (Panel B), egg production (Panel C), and feed conversion ratio (Panel D) of laying quails exposed to lead.**

No significant difference was found among the group fed by the diet supplemented with quercetin and the control group on performance parameters (Fig. 2, P>0.05). These results disagreed with those obtained by Liu et al. (2013) and Liu et al. (2014), who found that using 0.2 ve 0.4 g/kg quercetin improved laying rate and FCR in laying hens. The positive effects of quercetin on performance in laying hens may be attributed to hormone metabolism regulation phytoestrogen activity (Liu et al. 2013; Liu et al. 2014). Moreover, the enhanced performance may have occurred as a result of quercetin's positive effects on intestinal microbiota and the liver (Liu et al. 2013). In this study, there was no statistical difference between the control group and the quercetin group in terms of feed intake, egg weight, egg production, and feed conversion ratio (P>0.05). In this study, the supplemantation of quercetin to Pb group was found to improve egg weight, feed consumption and egg production but not statistically significant (P>0.05). This effect on performance may reflect very low bioavailability of quercetin and as a result of a lower digestibility. Moreover, it may be due to alterations of the composition of the cecal microflora by the quercetin. The different results between studies may also be explained by the different sources of quercetin used. At the end of the study, any statistical difference was not found between the groups in terms of survivability rate (Fig.3). Survivability rate was not affected by the quercetin and Pb level. The deaths occurred not associated with the trial.



Different letters indicate statistical differences among the groups. Each bar represents the mean and standard deviation. Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin.

Fig. 2: Effects of quercetin supplementation on feed intake (Panel A; P<0.01), egg weight (Panel B; P>0.05), egg production (Panel C; P<0.05), and feed conversion ratio (Panel D; P>0.05) of the experimental groups for 56 days.

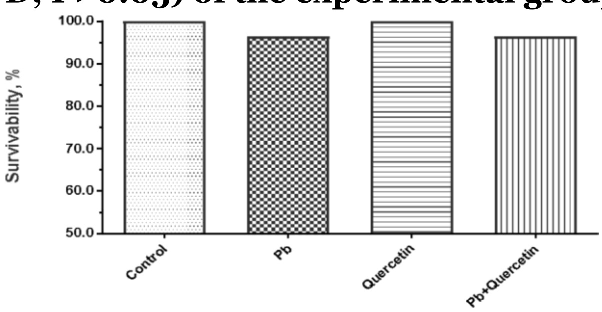


Fig. 3: The survivability rate of quails (%)

The present study showed that dietary Pb for 8 weeks was not affected Haugh unit, albumen index, yolk index, and shell weight (Table 3, P>0.05). Alagawany et al. (2018) found that dietary supplementation with Pb was not affected the egg quality criteria in laying quails. Faryadi et al. (2020) demonstrated that dietary Pb supplementation at 500 ppm for 5 weeks had no effect on egg yolk weight, albumin weight, eggshell weight, shape index, and shell thickness in Japanese quails. A significant decrease in shell thickness in the Pb group found in this study (Table 3, P<0.05) may be due to the suppression of calcium metabolism (Saly et al. 2004). Quercetin did not alter egg quality characteristics (P>0.05). This is in agreement with Liu et al. (2014), who found no significant difference in eggshell thickness and Haugh unit supplied with quercetin.

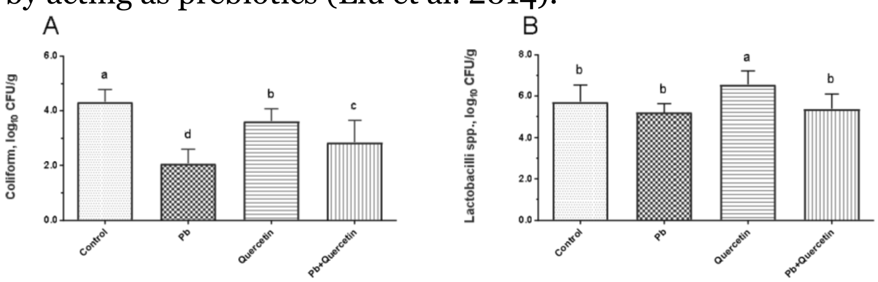
Table 3: Effects of quercetin on egg quality of the experimental groups

Egg Parameters	Control	Pb	Quercetin	Pb +Quercetin	P
Haugh unit, %	88.55±0.40	88.41±0.29	89.25±0.33	88.37±0.37	NS
Albumen index, %	10.00±0.13	9.81±0.13	10.01±0.16	9.90±0.17	NS

Yolk index, %	42.85±0.35	43.08±0.23	43.28±0.41	42.52±0.26	NS
Shell weight, g	0.94±0.01	0.92±0.01	0.92±0.01	0.92±0.01	NS
Shell thickness, mm	0.21±0.00[a]	0.20±0.00[b]	0.21±0.00[a]	0.20±0.00[ab]	*

Haugh unit, $100\times\log(H+7.571.7\times W^{0.37})$; H, albumen height, mm; W, egg weight, g.The data presents as mean and standard error. NS - non significant; NS: P>0.05, *: P<0.05. a, b: Mean values with different superscripts within a row differ significantly. Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin.

There is a close relationship between nutrition and intestinal microflora. The more a proportion of beneficial intestinal microorganisms, the better the development of animal health (Wang et al. 2018). In our study, 400 ppm of quercetin significantly increased the *Lactobacillus* in the cecum (Fig. 4B; P<0.05). The count of *Lactobacillus* in the quercetin group was significantly higher. In this study, the count of coliform in the group only treated with quercetin was significantly lower than in the control group (Fig. 4A; P<0.001). Dietary quercetin alleviated the increase of the coliform level in the cecum. Dong et al. (2020), similarly found that quercetin (400 ppm) supplemented broilers was able to promote *Lactobacillus* in the cecum. Wang et al. (2018) reported that supplementation with 0.2 g/kg quercetin significantly decreased the count of *E. coli* but significantly increased the count of *Lactobacillus*. These results indicated that quercetin may show a positive effect on the cecal microbiota by acting as prebiotics (Liu et al. 2014).

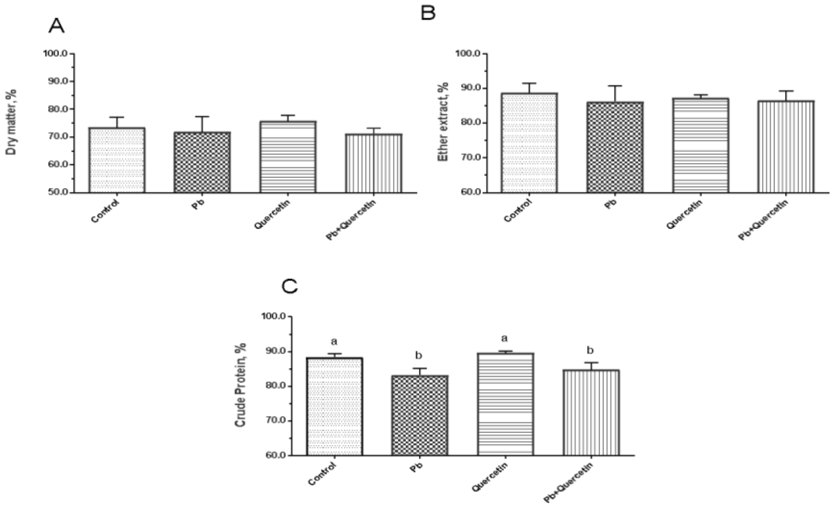


Different letters indicate statistical differences among the groups. Each bar represents the mean and standard deviation. Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin.

Fig. 4: The effects of quercetin on cecal bacteria populations (Coliform, Panel A; Lactobacillus, Panel B) of laying quails exposed to lead.

Gut microbiota alter the metabolism of heavy metals. It also regulates pH, and concentrations of detoxification enzymes or proteins. Pb induces oxidative stress, besides it affects the gut microbiota (Duan et al. 2020). Gao et al. (2017) found that the gut microbiome was disturbed by Pb exposure and Pb significantly reduced or inhibited the normal development of gut bacteria over time. We also found a change in gut microbiota in Pb exposed quail. The presence of Pb reduced the number of coliform bacteria in the cecum (P<0.05), but no significant changes in the number of *Lactobacillus* spp. (P>0.05). However, Jahromi et al. (2017) found that there was no significant difference in microbial population between the control and Pb-exposed birds.

Seven et al. (2012) observed that supplementation of 200 mg/kg Pb in the broilers did not affect dry matter and ether extract digestibility, but significantly reduced crude protein digestibility. Crude protein digestibility was affected by Pb supplementation (P<0.001). The decrease in crude protein digestibility could be due to oxidative stress which inhibits pancreatic enzymes activity (Seven et al. 2012). Azadbakht et al. (2017) reported that organic matter digestibility decreased in sheep chronically exposed to Pb contamination. These authors concluded that ether extract digestibility may be decreased due to interaction of Pb with long chain fatty acids at the gastro intestinal tract. The results showed that supplementation of quercetin did not significantly affect dry matter, ether extract, and crude protein digestibility (P>0.05). In agreement with our result in the present study, Park et al. (2020) showed in an in vivo study that dietary supplementation of quercetin in growing pigs tended to increase dry matter and nitrogen digestibility but had no significant effect on nutrient digestibility. This result may be related to the antioxidant effect of quercetin and the alteration of the intestinal microbiota (Kawabata et al. 2015). The inconsistent findings on nutrient digestibility may have been due to the relatively the dose of quercetin, animal species as well as diet types (Park et al. 2020).



Different letters indicate statistical differences among the groups. Each bar represents the mean and standard deviation. Pb: 100 mg/kg Lead, Quercetin: 400 mg/kg quercetin.

Fig. 5: The effects of quercetin on nutrient digestibility (Dry matter, Panel A; Ether extract, Panel B; Crude protein, Panel C) of laying quails exposed to lead. Conclusion

This research demonstrated that the Pb mitigated the performance, and nutrient digestibility. Quercetin can improve performance, nutrient digestibility, and the cecal microbial balance. The protective effect of quercetin may be associated with its an antioxidant and antibacterial activity. Therefore, the present results suggest that quercetin could be applied as an effective dietary strategy to ameliorate the toxic effects of Pb exposure in quails.

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