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Controlling Immunomodulation Effects of Deoxynivalenol Mycotoxins by NanoZinc Oxide and Probiotic in Broiler Chickens

Rasha Mahmoud Hamza Sayed-ElAhl[1]*, Atef Abdelaziz Hassan[1], Mogda Kamel Mansour[2], Azza Moustaffa Moustaffa Abdelmoteleb[2], and Ahmed Mohamed Attia El-Hamaky[1]

[1]*Department of Mycology and Mycotoxins, Animal Health Research Institute (AHRI), Agriculture Research Center (ARC), Giza, Egypt*
[2]*Department of Biochemistry, Toxicology and Feed Deficiency, Animal Health Research Institute (AHRI), Agricultural Research Center (ARC), Giza, Egypt*

*Corresponding author's E-mail: rasha_hamza2005@hotmail.com

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ABSTRACT

The elimination of adverse toxic effects of mycotoxins is currently the main strategy in animal production, particularly in poultry. The current study investigated the influence of chronic administration of deoxynivalenol on the health status, biochemical and immunological parameters of broiler chickens and the efficacy of ZnO-NPs and probiotics in preventing and treating the effect of toxicity. The experiment program lasted 6 weeks and was performed on a total of 60 broiler chickens aged 5 days, divided into six groups. Group 1 received healthy feed free of toxins, group 2 was fed with deoxynivalenol (DON), group 3 received Zinc Oxide nanoparticles (ZnO-NPs) and DON, group 4 had ZnO-NPs for 1 week, then DON was added for the remaining 5 weeks, group 5 was fed on ZnO-NPs, 1 g probiotic powder/kg of diet, and DON, group 6 had ZnO-NPs and 1 g probiotic powder/kg of diet for 1 week, then DON was added for 5 weeks. The used dose of ZnO-NPs was 50 ppm, and DON was 5 ppm in the diet. The intoxicated chickens showed adverse changes as increased pro-inflammatory cytokines, serum hepatic, and pancreatic enzymes, as well as decreased free amino acids. The supplementation of ZnO-NPs and/or probiotics improved all toxic changes resulting from DON toxicity, indicating that the metal nanoparticles and probiotics can be used together in poultry feed to avoid the addition of high doses of ZnO-NPs. Therefore, the use of 50 ppm of nanomaterial supplementation plus 1 g probiotic/ kg feed for the degradation of mycotoxins in poultry feed is recommended as it is safe and affordable.

Keywords: Deoxynivalenol, *Fusarium* spp., Nanoparticles, Poultry, Probiotic

INTRODUCTION

Worldwide morbidity and mortality from mycotoxicosis in humans and animals have a negative economic impact due to decreased health activities and output. Trichothecenes are secondary metabolites produced by *Fusarium* species, such as *F. oxysporum*, *F. poae*, and *F. graminearum* with specific references to trichothecene (T-2), deoxynivalenol (DON), and nivalenol (NIV, Chen et al., 2017; Springler et al., 2017).

Deoxynivalenol mycotoxin that has been discovered in food, may cause serious health problems and dangers to humans and animals. The toxicity occurs due to the conjugation of epoxide group with DNA subunits, which prevents protein synthesis and causes injury to human and animal tissues (Awad et al., 2013). Acute consumption of high doses of DON may cause several gastrointestinal diseases, such as emesis and diarrhea, while chronic consumption results in growth reduction and dangerous effects on digestive organs, as well as anemia, carcinogenesis, tremors, hemorrhage, dermatitis, pulmonary edema, immunosuppression, and hormonal imbalances in humans and animals (Rotter, 1996; Pestka, 2010). Poultry production is negatively affected by DON. The mentioned results are related to the dysfunction of the gastrointestinal tract, predisposition to infectious diseases

by the suppression of the cellular and humoral immune system through immune cell necrosis, serum immunoglobulins reduction, and spleen atrophy (Reddy et al., 2018; Riahi et al., 2020; Hassan et al., 2022).

Mycotoxins-management strategies have gained a lot of attention to preserve the health of animals and poultry. Although most conventional drugs are still used to treat these diseases, lower efficacy has been found due to the emergence of germ resistance, which requires finding innovative therapeutic medicines.

Nowadays, nanotechnology applications are of worldwide interest in all biomedical fields (Moghimi et al., 2005). Currently, zinc oxide nanoparticles (ZnO-NPs) have been shown to suppress microbial development, as well as enhancing appropriate antibacterial and antifungal action, and unique qualities, such as fewer environmental hazards and effectiveness in suppressing the growth of toxigenic fungi and their capacity to produce poisons (Reddy et al., 2007; Alghuthaymi et al., 2021). Moreover, immunity improvement, health promotion, and productivity in chickens can be seen as a result of ZnO-NPs (Lina et al., 2009). In addition, ZnO-NPs could couple with curcumin to diminish the viability of mycotoxin *Fusarium* spp. and inhibit the production of mycotoxins. A nanocomposite of ZnO-NPs and cinnamon oils may be able to remove *Fusarium* mycotoxins in animal feed (Gacem et al., 2021; Hassan et al., 2022).

Several studies have shown that probiotic formulations, particularly those containing *Lactobacillus* spp., have the potential to suppress fungal growth, and detoxify food and feed toxins (Fredua-Agyeman et al., 2017). The bio-detoxification of DON by microorganisms has been developed as a probiotic and has demonstrated significant toxin elimination benefits (Payros et al., 2016; Tiew et al., 2020; Chaudhari et al., 2022).

This study evaluated the toxicological effects of DON on broiler chickens and the efficacy of ZnO-NPs and/or probiotics in degrading the toxic effects of DON.

MATERIAL AND METHODS

Ethical approval

The experiment was approved by the Institutional Animal Health Research Ethics Committee, Egypt, and followed local laws and regulations (Vide Ref No. VMC/2014/IAEC/1046-73).

Feed samples

A total of 60 feed samples were collected from animal farms where chickens displayed toxicity symptoms, such as vomiting, diarrhea, anorexia, weight loss, and/or abrupt death. Every 500 g of feed sample was kept in a clean plastic bag and transported to the laboratory for analysis.

Zinc nanoparticles

The ZnO-NPs had a tiny particle of 50 nm in spherical forms that were prepared at the Nuclear Research Centre, Atomic Energy Authority, Egypt.

Probiotic vials

Micro-Procell vials each included 1 g of powder containing strains of *Lactobacillus plantarum* ($1x10^8$ CFU), and *Lactobacillus acidophilus* ($1x10^8$ CFU) were provided by Cheil Bio Co., Ltd, South Korea. *Saccharomyces cerevisiae* ($1x10^7$ CFU) with 0.5 g skim milk carrier was provided by Lallemand, SAS, France, under the name Levucell SB 10ME Titan (LSB).

Deoxynivalenol standard solution for mycotoxin detection using Thin Layer Chromatography

Mycotoxin's standard of DON was purchased from ALDRIK Sigma Chemical Company (St. Louis, USA).

Isolation and identification of *Fusarium* in feed samples

Under the aseptic process, 1 g of each feed sample was placed into sterile tubes containing 9 ml sterile distilled water to obtain tenfold serial dilution. Each ml of the serial dilutions was individually put into sterile petri dish plates and mixed with Sabouraud's dextrose agar containing 0.05 mg of chloramphenicol and incubated aerobically at 25°C for 3-5 days according to ISO (2008). *Fusarium* species were identified by macroscopic and microscopic features of mold colonies by Pitt and Hocking (2009).

Production of deoxynivalenol mycotoxin

The cultures of mycotoxigenic *Fusarium graminearum* isolates were cultivated on Potato Dextrose Agar (PDA) for 5-7 days at 25ºC. A total of 500 ml flasks with 100 gm of fine grounded yellow corn were autoclaved for 1 hour at 121ºC. Then, slant spores of each *Fusarium* species of 1 week old were added to the autoclaved flask and incubated for 4 weeks at 25-28ºC. The flasks were transferred to the refrigerator at 8-10ºC for 2 more weeks to produce a toxin (D'Mello et al., 1998). The produced trichothecenes mycotoxins were extracted and measured by thin-layer chromatography according to

studies by Kamimura et al. (1981) and Bottalico et al. (1985).

Chickens

A total of 60 broiler chickens aged 5 days from Dyerna farm, Giza, Egypt, were kept in clean cages while healthy feed and water *ad libitum* were available for 6 weeks. At 7 days of age, chickens were vaccinated against Newcastle and infectious bronchitis diseases using the HIPRAVAR Hitchner B1+H120 vaccine (HIPRA, Girona, Spain) via an intra-ocular route. At 10 days of age, the vaccination against avian influenza virus was done using CEVA C® NEW FLU - KEM inactivated H5N2 vaccines (CEVA, Giza, Egypt) via subcutaneous route.

Grouping

Chickens were divided into six groups. For the first group, healthy diet was available free of toxins, the second group received only DON and kept as intoxicated chickens, the third group was fed with ZnO-NPs and DON, the fourth group received ZnO-NPs for a week, then DON was added to the ZnO-NPs, the fifth group was fed on ZnO-NPs, 1 gm probiotic powder/kg of diet, and DON, finally, the sixth group received ZnO-NPs and 1 gm probiotic powder/kg of diet for a week, then DON was added. The utilized dosage of ZnO-NPs was 50 ppm and DON was 5 ppm in the diet. The experiment lasted 6 weeks following the studies of Yalcinkya et al. (2012) and Ahmadi et al. (2014).

Sampling

At the end of the experiment, 5 ml blood samples were collected from each chicken's brachial (wing) vein using a 20-gauge needle 1 inch in length. The serum was separated by centrifugation (SHengwin, USA) for 15 minutes and immediately frozen at -20ºC until analysis. According to AVMA Guidelines for the euthanasia of animals (Underwood and Anthony, 2020), Chickens were euthanized by cervical dislocation to collect livers and muscles from different groups to detect DON mycotoxin residues.

Serum biochemical parameters

Total protein was estimated according to Sonnen-Wirth and Jaret (1981). Assays for alanine aminotransferase (ALT), aspartate aminotransferase (AST), amylase, and lipase levels in serum were determined according to Lopez (2013). Malondialdehyde (MDA) and Glutathione (GSH) were determined following Ellman (1959), and Okhawa et al. (1979), respectively. Superoxide dismutase (SOD) was estimated according to Nishikimi et al. (1972). Amino acid analysis using the Amino Acid Analyzer sykam GmbH, Analytischer Messtechnik (Gewerbering 15, D_86922 Erosing, Germany) in the Genetic Research Center, Giza, Egypt.

Cytokines detection

Interleukin 6 (IL6) and the tumor necrotic factor-α (TNF-α) were measured by Nori chicken IL6 ELISA kits and Nori chicken TNF alpha ELISA kits, respectively (Genorise scientific, Inc., Pennsylvania, USA) according to the manufacturer's protocol**.**

Measurement of deoxynivalenol residues

Liver and muscle tissues were subjected to direct examination using a fluorometric assay to determine Deoxynivalenol residues, according to Kongkapan et al. (2016).

Statistical analysis

The obtained data were analyzed in SPSS, version 16 using ANOVA F-test and presented as mean ± standard error. The estimation of a significant difference was set at $p < 0.05$ (SPSS, 2007).

RESULTS AND DISCUSSION

Probiotics improve the health and immunity of animals infected with mycotoxins through inhibiting their cellular permeability and preventing their carcinogenic effects by enhancing their biodegradation (Ghadaksaz et al., 2022).

As presented in Table 1, *F. graminearum* produced higher levels of DON, T-2, Diacetoxy-scirpenol (DAS), and NIV resulting in higher total trichothecenes, compared to *F. sporotrichioides*. These toxins produced by *F. sporotrichioides* and *F. graminearum* negatively affect immunity and inhibit protein synthesis (Black et al., 1992; Osweiler, 2000). The higher level of DON in poultry feeds was reported as 5 mg/kg according to European Commission (2006). However, the lower levels of DON sometimes resulted in adverse effects on the suppression of chicken health status (Atanasova-Penichon et al., 2012; Lucke et al., 2017). Moreover, the administration of DON mycotoxins within the range of 5-15 ppm in the chicken feed negatively influenced productive performance, metabolic markers, and spleen cell growth, leading to DNA damage (Chen et al., 2017; Riahi et al., 2020). In addition, DON mycotoxin affects lipid peroxidation and DNA damage in chicken lymphocytes (Awad et al., 2012;

Ghareeb et al., 2015). The health of the internal organs of poultry, as well as biochemical and immunological markers, are influenced by the levels of 1-5 ppm, which causes jejunal and other digestive organ anomalies in hens.

Table 2 demonstrates a decrease in antioxidant activity by DON (G2), as measured by the significant reduction of SOD activity and GSH levels compared with (G1) ($p < 0.05$), and an increase of oxidative stress, measured by MDA levels, compared with control group ($p < 0.05$). Meanwhile, the administration of ZnO-NPs and/or probiotics ameliorated the damage caused by DON as shown by the elevated SOD, GSH, and lowered MDA levels compared with (G2). Antioxidant enzymes are critical in the defense against xenobiotic-induced oxidative damage. Deoxynivalenol is a polar molecule with three free hydroxyl groups (-OH), which contributes to oxidative stress by forming a high number of free radicals with an excess of oxidation markers, such as lipid peroxidation, MDA, and depleting antioxidants enzymes, including SOD and catalase, resulting in membrane and DNA damage (Nagy et al., 2005; Miloradovic et al., 2008; Wu et al., 2014).

Dawei et al. (2009) and Hu et al. (2012) found the ability of ZnO-NPs to scavenge free radicals and suppress the incidence of cell damage and carcinogenesis. Furthermore, the administration of ZnO-NPs enhanced the effectiveness of digestion and hence the availability of body nutrients. Moreover, Gacem et al. (2021), Mandal (2021), and Singh et al. (2021) confirmed that ZnO-NPs significantly elevated the antioxidant activity of cell membrane enzymes and reduced MDA. Probiotic strains that can restrict excessive levels of reactive radicals *in vivo* may prevent and treat various disorders related to oxidative stress. Probiotics elevate antioxidant defenses by producing vitamins and releasing GSH that are absorbed and dispersed throughout the body (Spyropoulos et al., 2011; Kushkevych and Jampílek, 2021; Hassan et al., 2022).

Table 1. Prevalence of trichothecenes mycotoxins produced by the isolated *Fusarium* spp. from poultry feeds

Trichothecene mycotoxins (ppm) / *Fusarium* spp. (5 strains of each)	DON (Mean ± SE)	T-2, DAS, NIV (Mean ± SE)	Total trichothecene (Mean ±SE)
F. graminearum	22.5 ± 0.60	12.5 ± 1.5	35.0 ± 2.2
F. sporotrichioides	13.5 ± 1.9	7.5 ± 0.83	21 ± 1.95

SE: Standard error, *F*: *Fusarium*, DON: Deoxynivalenol, T-2: Trichothecene, DAS: Diacetoxy-scirpenol, NIV: Nivalenol.

Table 2. Effects of dietary supplementation of ZnO-NPs and/or probiotic on oxidative stress and antioxidant activities in broiler chickens contaminated with deoxynivalenol

Groups	MDA (nmol/ml)	GSH (u/ml)	SOD (u/ml)
G1	$1.09^{c} \pm 0.04$	$6.90^{a} \pm 0.06$	$55.52^{a} \pm 1.02$
G2	$1.70^{a} \pm 0.08$	$4.83^{c} \pm 0.13$	$28.00^{d} \pm 1.89$
G3	$1.38^{b} \pm 0.03$	$5.53^{b} \pm 0.13$	$30.33^{c} \pm 1.45$
G4	$1.63^{a} \pm 0.08$	$5.18^{bc} \pm 0.23$	$34.30^{c} \pm 1.93$
G5	$1.29^{bc} \pm 0.12$	$5.68^{b} \pm 0.18$	$38.33^{bc} \pm 2.73$
G6	$1.28^{bc} \pm 0.03$	$5.66^{b} \pm 0.17$	$44.33^{b} \pm 5.99$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for 1 week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles, MDA: malondialdehyde, GSH: Glutathione, SOD: superoxide dismutase.

Table 3. Effects of dietary supplementation of ZnO-NPs and/or probiotic on total protein and some enzyme activities in broiler chickens contaminated with deoxynivalenol

Groups	TP (g/dl)	ALT (U/l)	AST (U/l)	Amylase (U/l)	Lipase (U/l)
G1	$4.10^{a} \pm 0.07$	$29.00^{d} \pm 1.53$	$54.00^{d} \pm 0.58$	$881.67^{e} \pm 1.76$	$1.05^{c} \pm 0.03$
G2	$3.07^{c} \pm 0.12$	$54.67^{a} \pm 2.60$	$93.70^{a} \pm 1.82$	$1244.65^{a} \pm 5.53$	$1.25^{a} \pm 0.05$
G3	$3.58^{b} \pm 0.06$	$40.00^{b} \pm 0.58$	$84.33^{b} \pm 1.67$	$1002.67^{c} \pm 29.21$	$1.14^{bc} \pm 0.01$
G4	$3.53^{b} \pm 0.03$	$41.67^{b} \pm 0.33$	$82.63^{b} \pm 0.82$	$1115.67^{b} \pm 0.67$	$1.19^{ab} \pm 0.04$
G5	$3.71^{b} \pm 0.03$	$35.33^{c} \pm 1.20$	$72.37^{c} \pm 0.74$	$975.00^{cd} \pm 4.58$	$1.13^{bc} \pm 0.01$
G6	$3.72^{b} \pm 0.06$	$34.67^{c} \pm 1.45$	$74.67^{c} \pm 2.03$	$936.32^{d} \pm 18.18$	$1.06^{c} \pm 0.01$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for 1 week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles, TP: Total protein, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase.

Table 3 presents the total serum protein (TP), ALT, and AST as indicators of chicken metabolism and visceral organs' health. According to Pestka, (2010), DON is a powerful protein synthesis inhibitor, causing protein turnover disruption even at low concentrations. Compared to the control group, serum ALT and AST levels have also been found to be sensitive markers of liver damage since a rise in these values in the DON treatment groups indicates leakage of injured hepatocytes following DON intake (Nyblom et al., 2004).

The pancreatic enzyme showed a significant rise in serum amylase and lipase activity after DON intake ($p < 0.05$). Similarly, according to previous studies, DON enhanced the activity of pancreatic chymotrypsin, amylase, and lipase (Richardson and Hamilton, 1987; Matur et al., 2010). On the contrary, Osborne et al. (1982) believe that DON ingestion causes malabsorption, defined as steatorrhea, hypocarotenoidemy, and reduces bile, pancreatic lipase, trypsin, and amylase. The administration of ZnO-NPs to the contaminated groups resulted in lower levels of liver function indicators, such as ALT and AST, compared to the non-treated group (Table 3). These findings agree with Ahmadi et al. (2014), who demonstrated that dietary ZnO-NPs decreased serum ALT in broiler chickens. ZnO-NPs also significantly reduced amylase and lipase levels ($p < 0.05$), resulting in a lower histopathological damage score in both the pancreas and the liver tissues.

Table 4 presents that the mycotoxicated group (G2) had a significant elevation of the pro-inflammatory cytokines, including both interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) compared with G1 ($p < 0.05$). Cytokines produced by immune cells have significant functions in immune response (Giansanti et al., 2006). Similar findings to the current results were reported by Awad et al. (2013) and Hendrayani et al. (2016), who found that the administration of deoxynivalenol contaminated diet in chickens increased serum levels of IL-6 and TNF-α. Another study indicated that exposure to DON potentiated inflammatory gene expression and the chronic doses resulted in necrosis and death cells (Shi et al., 2009; Wu et al., 2015; Peng et al., 2019). Furthermore, ZnO-NPs and/or probiotics treatment in groups 3, 4, 5, and 6 showed anti-inflammatory activity by IL-6, and TNF-α. The results are consistent with earlier research by El-Bahr et al. (2020), Mandal (2021), and Sakthivel et al. (2021), who found that ZnO-NPs increased the levels of antioxidant enzymes, including SOD, catalase, and glutathione peroxidase and decreased IL-6 and TNF-α cytokines in liver and brain tissues. The current study revealed lower levels of free amino acids in G2 (Table 5). After DON exposure, the observed initial adverse effects included reduced feed intake, emesis, diarrhea, and anorexia (Pestka and Smolinski, 2005). Moreover, DON exposure negatively affected the absorption and metabolism of different biological and intestinal flora resulting in adverse toxic effects (Awad and Zentek, 2015). It was suggested by Wu et al. (2014) that reduced levels of amino acid in contaminated chicks with DON are due to intestinal barrier dysfunction. Table 5 shows that contaminated chickens with DON (G2) adversely affected the health status of chickens by reducing levels of amino acids. However, the supplementation of ZnO-NPs and/or probiotics improved amino acids levels in groups 3, 4, 5, and 6. The amino acids are essential for protein synthesis, and reduced absorption because of DON toxicity resulted in losses in the productivity of poultry (Wu et al., 2015).

The administration of ZnO-NPs in group 3 manifested significant increase in serum amino acid of chickens, compared to the second group ($p < 0.05$). According to Zhang et al. (2018), leucine, isoleucine, and proline were elevated by ZnO-NPs, compared to the normal control. The serum lysine content in the ZnO-NPs and/or probiotic groups increased in comparison with the second contaminated group due to increase amino acid absorption in treated chickens (Wu et al., 2015). The present study detected that the probiotic administration in contaminated chicken feed helped the chicken overcome the toxic effects of DON on some vital organs, such as liver, this agreed with (Marzouk et al., 2008; Mehrim, 2009). Recently, it has been reported that the probiotic preparation initiated the immune function of phagocytosis and epithelial cells against DON toxicity and microbial infection (Vinderola and Ritieni, 2015; Chaudhari et al., 2022; Hassan et al., 2022). Moreover, the elimination of DON by the supplementation of probiotic strains in the feed may be due to the occurrence of malabsorption of toxins in the gastrointestinal tract of treated livestock and reduced oxidative stress and inflammatory response (Chen et al., 2013; Zhao et al., 2013; Vila-Donat et al., 2018).

Previous studies evaluated the elimination of *Fusarium* toxins (Trichothecenes; DON) from food using *bacillus* probiotics (Zhao et al., 2016; Wang et al., 2020; Thipe et al., 2022).

According to Table 6, the second group received only DON and had the highest levels of DON in liver and muscle tissues. The third group received ZnO-NPs with DON and had better elimination of DON in the liver and muscles. The trials of administration of ZnO-NPs and or probiotics in the fourth and sixth groups before the addition of DON showed lower levels of detoxification. However, the residues of DON in the liver after treatment were still within the permissible limits, as reported by Escrivá et al. (2017).

Table 4. Effects of dietary supplementation of ZnO-NPs and/or probiotic IL- 6 and TNF- α: of broiler chickens contaminated with deoxynivalenol

Parameters	G1	G2	G3	G4	G5	G6
IL- 6 (ng/l)	$121.51^{d} \pm 2.19$	$147.83^{a} \pm 3.63$	$130.33^{bc} \pm 3.38$	$137.33^{b} \pm 1.67$	$128.33^{c} \pm 1.45$	$135.00^{bc} \pm 2.31$
TNF- α (ng/l)	$69.67^{d} \pm 2.03$	$112.33^{a} \pm 6.94$	$98.00^{ab} \pm 6.51$	$109.67^{a} \pm 4.84$	$87.67^{b} \pm 4.70$	$104.67^{ab} \pm 5.17$

Data are represented as mean value ± Standard error. Values in the same row with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic. ZnO-NPs: Zinc oxide nanoparticles. IL- 6: Interleukin-6, TNF- α: Tumor necrosis factor-α

Table 5. Effects of dietary supplementation of ZnO-NPs and/or probiotic on some serum amino acids (g/dl) of broiler chickens contaminated with deoxynivalenol

Groups	Lysine	Methionine	Leucine	Iso-leucine	Arginine	Proline
G1	$2.09^{a} \pm 0.01$	$1.33^{a} \pm 0.03$	$2.43^{a} \pm 0.05$	$1.72^{a} \pm 0.03$	$2.62^{a} \pm 0.03$	$1.00^{a} \pm 0.01$
G2	$1.61^{c} \pm 0.05$	$1.11^{c} \pm 0.01$	$2.05^{b} \pm 0.03$	$1.21^{d} \pm 0.02$	$2.14^{cd} \pm 0.04$	$0.65^{d} \pm 0.04$
G3	$1.79^{b} \pm 0.03$	$1.26^{ab} \pm 0.05$	$2.18^{b} \pm 0.02$	$1.33^{c} \pm 0.00$	$2.21^{c} \pm 0.01$	$0.90^{b} \pm 0.01$
G4	$1.70^{bc} \pm 0.02$	$1.13^{c} \pm 0.02$	$2.09^{b} \pm 0.01$	$1.31^{c} \pm 0.01$	$2.30^{bc} \pm 0.04$	$0.80^{c} \pm 0.03$
G5	$1.74^{b} \pm 0.06$	$1.24^{b} \pm 0.01$	$2.16^{b} \pm 0.03$	$1.37^{c} \pm 0.03$	$2.38^{b} \pm 0.03$	$0.91^{b} \pm 0.01$
G6	$1.79^{b} \pm 0.03$	$1.22^{b} \pm 0.02$	$2.15^{b} \pm 0.12$	$1.54^{b} \pm 0.06$	$2.30^{bc} \pm 0.04$	$0.95^{ab} \pm 0.02$

Data are represented as mean value ± Standard error. Values in the same column with the different superscript letters are significantly different ($P < 0.05$). G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic, ZnO-NPs: Zinc oxide nanoparticles

Table 6. Levels of deoxynivalenol residues in the liver and muscles (ppb) of contaminated broiler chickens

Organs	G1	G2	G3	G4	G5	G6
Liver	ND	1.56 ± 0.14	0.51 ± 0.20	0.31 ± 0.15	1.1 ± 0.27	1.3 ± 0.20
Muscles	ND	1.6 ± 0.25	0.05 ± 0.01	0.03 ± 0.01	0.06 ± 0.02	0.03 ± 0.00

G1: Control group, G2: Deoxynivalenol (DON) intoxicated group, G3: ZnO-NPs + DON group, G4: ZnO-NPs only for 1 week then DON + ZnO-NPs, G5: ZnO-NPs + DON + probiotic, G6: ZnO-NPs + probiotic only for one week then DON + ZnO-NPs + probiotic DON. ZnO-NPs: Zinc oxide nanoparticles, ND: Not detectable

CONCLUSION

According to the findings, *Fusarium* species isolated from chicken feeds produced significant amounts of Trichothecenes mycotoxins, particularly DON. In the present investigation, this toxin has a detrimental effect on the health status, immunological state, and output of chickens. Furthermore, supplementation of ZnO-NPs, either alone or in combination with the probiotic, showed considerable potential in eliminating the negative activity of DON in broiler chickens. As a result, supplementation of ZnO-NPs and probiotic can significantly improve poultry and animal health, as well as their products such as meat, eggs, milk, wool, and leather. Finally, it is recommended to conduct future studies on the addition of probiotics to ZnO-NPs for the degradation of mycotoxins and lowering the used doses of nanomaterials to avoid their toxic levels.

DECLARATIONS

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Authors' contribution

Rasha Sayed-ElAhl designed the research. Rasha Sayed-ElAhl, Atef Hassan, Mogda Mansour, Azza Abdelmoteleb, and Ahmed El Hamaky performed the experimental duties of this study and analyzed the data. Mogda Mansour did the statistical analyses. Atef Hassan and Mogda Mansour wrote the draft of the manuscript.

Mogda Mansour, Rasha Sayed-ElAhl, Azza Abdelmoteleb, and Ahmed El Hamaky have taken part in the revision of the manuscript. All authors read and approved the final version of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors investigated ethical issues such as plagiarism, permission to publish, malfeasance, data falsification and/or fabrication, double publishing and/or submission, and redundancies.

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