Effects of Low-Dose Zearalenone and Adsorbent on Growth Performance, Serum Biochemical Indices, and Antioxidant Indices of Growing Laying Hens (Postprint)

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Abstract

This study aimed to investigate the effects of low-dose zearalenone (ZEA) on growth performance, serum biochemical indices, and antioxidant indices of growing laying hens, and to evaluate the detoxification efficacy of a modified montmorillonite adsorbent (Calibrin-A, CA). A total of 720 70-day-old Hy-Line Brown laying hens were randomly divided into 4 groups with 5 replicates per group and 36 hens per replicate. The control group was fed a basal diet, experimental group 1 was supplemented with 0.15% CA on top of the basal diet, experimental group 2 replaced the corn protein meal in the basal diet with naturally moldy corn protein meal and adjusted the dietary toxin level with pure ZEA (ZEA = 0.4 mg/kg), and experimental group 3 was supplemented with 0.15% CA on top of experimental group 2. The pre-trial period was 7 days, and the formal trial period was 49 days. The results showed: 1) Low-dose ZEA and CA had no significant effect on the growth performance of growing laying hens ($P > 0.05$). 2) Low-dose ZEA significantly increased the concentrations of low-density lipoprotein (LDL), cholesterol, and uric acid in serum on day 25 of the experiment ($P < 0.05$), and CA supplementation significantly decreased the concentrations of LDL, cholesterol, and uric acid in serum ($P < 0.05$). 3) Low-dose ZEA significantly decreased the activities of glutathione peroxidase (GSH-Px) (on days 25 and 47) and total superoxide dismutase (T-SOD) (on day 47) in serum ($P < 0.05$), and significantly increased the content of malondialdehyde (MDA) in serum (on days 25 and 47) ($P < 0.05$); compared with the low-dose ZEA group, CA supplementation significantly increased the activities of GSH-Px (on day 47) and T-SOD (on day 47) in serum ($P < 0.05$), and significantly decreased the content of MDA in serum (on days 25 and 47) ($P < 0.05$). In conclusion, 0.4 mg/kg ZEA in the diet did not affect the growth performance of growing laying

hens, but significantly affected their serum biochemical and antioxidant indices, and CA supplementation in the ZEA group had a significant ameliorative effect on serum indices.

Full Text

Effects of Low-Dose Zearalenone and Adsorbent on Growth Performance, Serum Biochemical Parameters, and Antioxidant Indices of Growing-Laying Hens

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Abstract

This experiment was conducted to investigate the effects of low-dose zearalenone (ZEA) on growth performance, serum biochemical parameters, and antioxidant indices of growing-laying hens, and to evaluate the detoxification efficacy of a modified montmorillonite adsorbent (Calibrin-A, CA). A total of 720 Hy-Line Brown laying hens aged 70 days were randomly allocated into 4 groups with 5 replicates per group and 36 hens per replicate. The control group was fed a basal diet, while experimental group 1 received the basal diet supplemented with 0.15% CA. Experimental group 2 was fed a diet in which naturally contaminated corn gluten meal replaced the basal corn gluten meal, with purified ZEA added to achieve a dietary toxin level of 0.4 mg/kg. Experimental group 3 received the same diet as group 2 supplemented with 0.15% CA. The experiment consisted of a 7-day pre-trial period followed by a 49-day formal trial period. The results showed that: (1) Low-dose ZEA and CA had no significant effects on the growth performance of growing-laying hens ($P>0.05$). (2) Low-dose ZEA significantly increased serum concentrations of low-density lipoprotein (LDL), cholesterol, and uric acid on day 25 ($P<0.05$), and CA supplementation significantly reduced these serum LDL, cholesterol, and uric acid concentrations ($P<0.05$). (3) Low-dose ZEA significantly decreased serum glutathione peroxidase (GSH-Px) activity (on days 25 and 47) and total superoxide dismutase (T-SOD) activity (on day 47) ($P<0.05$), while significantly increasing serum malondialdehyde (MDA) content (on days 25 and 47) ($P<0.05$). Compared with the low-dose ZEA group, CA supplementation significantly increased serum GSH-Px (on day 47) and T-SOD (on day 47) activities ($P<0.05$) and significantly decreased serum MDA content (on days 25 and 47) ($P<0.05$). These findings indicate that dietary ZEA at 0.4 mg/kg did not affect growth performance but significantly impacted serum biochemical and antioxidant indices in growing-laying hens, and CA addition to ZEA-contaminated diets produced significant ameliorative effects on these serum parameters.

Keywords: growing-laying hens; zearalenone; adsorbent; growth performance; metabolism; antioxidant

Introduction

Zearalenone (ZEA), also known as F-2 toxin [1], is a mycoestrogen produced by *Fusarium* species that contaminates corn particularly severely in northern China [2]. Research has demonstrated that ZEA and its metabolites can cause mycotoxicosis in many animal species [3-5], with pigs being especially sensitive. Low-dose ZEA (3 mg/kg) does not affect growth performance in weaned piglets [6-7], and one study reported that 1 mg/kg ZEA in feed increased average daily gain (ADG) without affecting average daily feed intake (ADFI) or feed conversion ratio (FCR) [8]. However, ZEA at 1.5 mg/kg body weight can impair hepatocyte function and alter blood parameters in female rats [9]. ZEA-induced hepatic stress suppresses lipid secretion [10-11], and at concentrations of 1-10 μg/mL, ZEA reduces glutathione (GSH) content, decreases superoxide dismutase (SOD) activity, and increases malondialdehyde (MDA) production in primary cultured intestinal epithelial cells in a dose-dependent manner [12]. The most effective method for adsorbing mycotoxins in animal feed is supplementation with montmorillonite adsorbents, which have been proven effective for ZEA adsorption in vitro and in animal trials with rats and pigs [13-15].

While numerous studies on ZEA have focused on pigs and mice, research on the toxic effects of low-dose ZEA (0.4 mg/kg) in growing-laying hens has not been reported. This experiment was designed to investigate the effects of low-dose ZEA on growth performance, serum biochemical parameters, and antioxidant indices in growing-laying hens, and to evaluate the ameliorative effects of modified montmorillonite adsorbent on ZEA toxicity, providing a reference basis for egg production.

Materials and Methods

1.1 Experimental Materials **Zearalenone:** Prior to the experiment, 121 different corn products were sampled from four cities in Shandong Province (Tai'an, Jinan, Liaocheng, and Heze) for mycotoxin analysis. Naturally contaminated corn gluten meal containing only low-dose ZEA (crude protein content 51.1%; ZEA content 1.3 mg/kg) was selected as the experimental material, and purified ZEA (produced by Fermentek, Israel, with guaranteed purity of 98%) was used to adjust the dietary ZEA concentration to 0.4 mg/kg.

Adsorbent (Calibrin-A, CA): A thermally modified montmorillonite adsorbent provided by Oil-Dri Corporation, USA.

1.2 Experimental Animals and Management A total of 720 healthy Hy-Line Brown laying hens aged 70 days with an average body weight of (1.07$±$0.02) kg were randomly divided into 4 groups with 5 replicates per

group and 36 hens per replicate, with no significant differences in initial body weight among groups (P>0.05). The control group (Contr.) received the basal diet, experimental group 1 (Contr.+CA) received the basal diet supplemented with 0.15% CA, experimental group 2 (Mycot.) was fed a diet formulated by completely replacing the corn gluten meal in the basal diet with naturally contaminated corn gluten meal (ZEA=0.4 mg/kg), and experimental group 3 (Mycot.+CA) received the same diet as group 2 supplemented with 0.15% CA. The experiment included a 7-day pre-trial period and a 49-day formal trial period. The basal diet was formulated according to NRC (1994) standards, with composition and nutrient levels shown in Table 1 . Experimental hens were housed in two-tier step cages with free access to feed and water, and were vaccinated according to normal immunization procedures.

1.3 Blood Sample Collection and Processing On days 25 and 47 of the experiment, 6 hens were randomly selected from each replicate for wing vein blood collection. Blood samples collected in vacuum coagulation-promoting tubes were placed at a 30° angle in a 37°C water bath for 10 minutes, then centrifuged at 3,000 r/min for 10 minutes to separate serum, which was stored at -20°C for subsequent determination of serum biochemical and antioxidant indices.

1.4.1 Dietary Toxin Detection ZEA and aflatoxin contents were determined by liquid chromatography with fluorescence detection after immunoaffinity column cleanup, using external standard quantification. Fumonisin and deoxynivalenol contents were determined by high-performance liquid chromatography-tandem mass spectrometry with UV detection after immunoaffinity cleanup, using external standard quantification. The detection limits for ZEA, aflatoxin, fumonisin, and deoxynivalenol were 0.1 mg/kg, 1.0 g/kg, 0.25 mg/kg, and 0.1 mg/kg, respectively. The measured mycotoxin contents in each experimental diet are shown in Table 2 .

1.4.2 Growth Performance Indices Feed intake and body weight were recorded weekly on a replicate basis throughout the trial period for calculating average daily gain (ADG), average daily feed intake (ADFI), and feed-to-gain ratio (F/G).

1.4.3 Serum Biochemical Parameters Serum biochemical parameters including total protein, cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), and uric acid concentrations were determined using a COBAS MIRA Plus automatic biochemical analyzer. All reagent kits were purchased from Nanjing Jiancheng Bioengineering Institute.

1.4.4 Serum Antioxidant Indices Total superoxide dismutase (T-SOD) activity was determined by the xanthine oxidase method (hydroxylamine method), glutathione peroxidase (GSH-Px) activity by chemical colorimetry, and MDA

content by colorimetry. T-SOD activity assay kit (A001-1), GSH-Px activity assay kit (A005), and MDA content assay kit (A003) were purchased from Nanjing Jiancheng Bioengineering Institute.

1.5 Statistical Analysis Experimental data were analyzed using SAS 9.2 software. Two-factor ANOVA was used for statistical analysis, and Duncan' s multiple range test was applied for pairwise comparisons. The significance level was set at $P<0.05$.

Results

2.1 Growth Performance The effects of low-dose ZEA on growth performance of growing-laying hens are shown in Table 3 . Low-dose ZEA had no significant effects on ADG, ADFI, or F/G in growing-laying hens ($P>0.05$). CA supplementation also did not significantly affect growth performance ($P>0.05$). No significant interaction between ZEA and CA was observed for any growth performance parameter ($P>0.05$).

2.2 Serum Biochemical Parameters As shown in Table 4 , on day 25, serum LDL, cholesterol, and uric acid concentrations were significantly higher in the low-dose ZEA group compared with the control group ($P<0.05$). CA supplementation significantly reduced serum LDL, cholesterol, and uric acid concentrations in the ZEA group ($P<0.05$), while total protein, triglyceride, and HDL concentrations showed no significant differences ($P>0.05$). Neither low-dose ZEA nor CA significantly affected serum biochemical parameters on day 47 ($P>0.05$). A significant interaction between ZEA and CA was observed for serum cholesterol concentration on day 25 ($P<0.05$).

2.3 Serum Antioxidant Indices As shown in Table 5 , low-dose ZEA significantly decreased serum GSH-Px activity (on days 25 and 47) and T-SOD activity (on day 47) ($P<0.05$), while significantly increasing serum MDA content (on days 25 and 47) ($P<0.05$). Compared with the low-dose ZEA group, CA supplementation significantly increased serum GSH-Px (on day 47) and T-SOD (on day 47) activities and significantly decreased serum MDA content (on days 25 and 47) ($P<0.05$). CA supplementation alone had no significant effects on any indices compared with the control group ($P>0.05$). A significant interaction between ZEA and CA was observed for serum MDA content on day 47 ($P<0.05$).

Regarding ZEA research, some studies have used direct addition of purified ZEA toxin [14], while others have used naturally contaminated feed with known ZEA content [16]. However, naturally contaminated feed often contains one or more toxins besides ZEA, which may confound studies on ZEA toxicity. In this experiment, the feed was formulated using naturally contaminated corn gluten meal containing only ZEA and purified ZEA toxin. Analysis confirmed that, apart from low-dose ZEA, only aflatoxin was present at levels far below feed

hygiene standard limits, making the experimental diets suitable for investigating ZEA toxicity in growing-laying hens.

Discussion

3.1 Effects of Low-Dose ZEA on Growth Performance of Growing-Laying Hens The present results indicate that low-dose ZEA from naturally contaminated corn gluten meal had no significant effects on ADFI, ADG, or F/G in growing-laying hens. Marin et al. [17] reported that feeding weaned piglets a complete diet containing ZEA (316 g/kg) had no effect on body weight, ADG, or ADFI. Nikaido et al. [18] found that ZEA (0.1 or 10 mg/kg BW) did not alter body weight in pre-pubertal female rats, consistent with our findings. However, other studies have shown that increasing dietary ZEA levels decreased ADG, ADFI, and FCR in piglets [19], with feed efficiency increasing linearly with ZEA supplementation [20]. These discrepancies may be related to ZEA dosage, experimental duration, and animal species used.

3.2 Effects of Low-Dose ZEA on Serum Biochemical Parameters of Growing-Laying Hens This study demonstrated that the toxin group containing 0.4 mg/kg ZEA exhibited elevated serum cholesterol in growing-laying hens, presumably due to hepatocyte damage. This is consistent with Sun Meile et al. [21], who reported ZEA-induced damage to rat hepatocytes in vitro. Ojeda [22] also confirmed the anti-estrogenic effects of ZEA, while estrogen can increase triglyceride synthesis and fat deposition while decreasing circulating cholesterol levels. On day 25, the ZEA group showed increased uric acid, which is primarily influenced by kidney function, protein intake, and catabolism. This suggests that low-dose ZEA also exerts some nephrotoxicity in growing-laying hens, consistent with findings in rats [23], though further renal immunohistochemical verification is needed. However, as the experiment progressed (day 47), the effects of ZEA (0.4 mg/kg) on serum biochemical parameters were no longer significant, suggesting that under these experimental conditions, growing-laying hens may have developed self-regulatory mechanisms against ZEA, though this requires confirmation through repeated trials.

3.3 Effects of Low-Dose ZEA on Serum Antioxidant Indices of Growing-Laying Hens During normal metabolic processes, biological organisms produce superoxide anion radicals ($\bullet O_2^-$), which trigger lipid peroxidation reactions that affect the oxidative-antioxidant balance and may alter important metabolic processes such as cell membrane metabolism, protein biosynthesis, and glycolysis [24]. T-SOD, GSH-Px, and catalase (CAT) are the primary endogenous antioxidant enzymes that scavenge free radicals in cells [25]. T-SOD is widely distributed in various tissues and can eliminate O_2^-, while MDA is the final product of lipid peroxidation [22]. Therefore, normal GSH-Px and T-SOD activities and MDA content are crucial for maintaining oxidative balance. Studies have confirmed that ZEA causes significant oxidative damage [26-29], with piglets fed 2.0 and 3.2 mg/kg ZEA showing significantly

lower serum and hepatic SOD and GSH-Px activities and higher MDA content than controls [16]. The present study found that by day 25, the 0.4 mg/kg ZEA group exhibited significantly higher serum MDA content and lower GSH-Px activity compared with controls. As the experiment progressed, the 0.4 mg/kg ZEA group showed significantly lower T-SOD and GSH-Px activities and higher MDA content, consistent with the piglet studies mentioned above. Some research has also shown that naturally contaminated corn can increase hepatic SOD activity [30], possibly because multiple toxins in naturally contaminated corn stimulate self-regulatory mechanisms that produce more SOD to eliminate increased O_2^-.

3.4 Effects of Modified Montmorillonite Adsorbent on Growing-Laying Hens Previous studies have shown that dietary supplementation with zeolite or activated carbon does not affect growth performance in weaned piglets [31-32]. Similarly, this experiment demonstrated that 0.15% CA supplementation had no significant effect on growth performance in growing-laying hens, presumably because this level of CA supplementation does not affect intestinal function or nutrient absorption and metabolism. Yang et al. [33] reported that 0.5% montmorillonite adsorbent supplementation in broiler diets did not affect serum biochemical parameters. The present study also showed that 0.15% CA supplementation alone had no significant effect on hepatic and blood metabolism in growing-laying hens. However, CA supplementation significantly improved serum LDL, cholesterol, and uric acid levels in the contaminated group on day 25. Previous research indicated that adding 1 or 2 kg/t of modified montmorillonite Calibrin-Z (CZ) to low-dose ZEA (1.3 mg/kg) diets did not significantly improve serum antioxidant enzymes or MDA in weaned piglets, though a trend toward improvement was observed, and 4 kg/t CZ was required for significant effects [15]. This experiment confirmed that 0.15% CA supplementation in low-dose ZEA diets increased serum GSH-Px and T-SOD activities and decreased MDA content in growing-laying hens, exerting clear effects on oxidative balance, consistent with the weaned piglet studies.

Conclusions

Under the conditions of this experiment:

1. ZEA at 0.4 mg/kg had no significant effects on growth performance of growing-laying hens.
2. ZEA at 0.4 mg/kg increased serum LDL, cholesterol, and uric acid concentrations in growing-laying hens, though the toxicity diminished with prolonged experimental duration.
3. ZEA at 0.4 mg/kg decreased serum GSH-Px and T-SOD activities and increased MDA content, with toxicity accumulating over time.
4. Compared with the control group, 0.15% CA supplementation did not affect growth performance, serum biochemical parameters, or antioxidant

indices. However, compared with the ZEA group, 0.15% CA supplementation significantly ameliorated serum biochemical and antioxidant parameters in growing-laying hens.

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Note: Figure translations are in progress. See original paper for figures.