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AN EVALUATION OF THE SUPPLEMENTATION OF DIETARY-MODIFIED PALYGORSKITE ON GROWTH PERFORMANCE, ZEARALENONE RESIDUE, SERUM METABOLITES, AND ANTIOXIDANT CAPACITIES IN BROILERS FED A ZEARALENONE-CONTAMINATED DIET

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Abstract—Zearalenone (ZEA), a common contaminant in food and feedstuffs, threatens human and animal health. The present study aimed to investigate the protective effects of modified palygorskite (MPal), a ZEA-targeted adsorbent, on broilers (young chickens) fed a ZEA-contaminated diet. Broilers were subjected to one of three treatments for a period of 42 days: a basal diet (control group), a ZEA-contaminated diet, and a ZEA-contaminated diet supplemented with 1 g/kg of MPal. Blood was collected for serum metabolite assay, and liver and kidney were sampled to determine ZEA residue and antioxidant-related parameters, using commercial spectrophotometric kits. Compared with the basal diet, the ZEA-contaminated diet resulted in compromised growth performance (reduced daily gain and feed intake during finisher period), disordered relative liver weight (decreased at 21 days but increased at 42 days), increased ZEA residue in liver and kidney, abnormal serum metabolites (decreased total protein content but increased alanine aminotransferase activity at 21 and 42 days, reduced albumin content at 21 days, and elevated aspartate aminotransferase activity at 42 days), and disrupted antioxidant capacities of broilers (increased total superoxide dismutase (T-SOD) activity in liver at 21 and 42 days, decreased T-SOD activity in kidney at 21 and 42 days, and in serum at 42 days, greater malondialdehyde accumulation in liver and kidney at 42 days, and lower glutathione content in kidney at 21 days). The adverse consequences resulting from the ZEA-contaminated diet were relieved by the supplementation of MPal (except albumin concentration in serum and T-SOD activity in liver at 21 days), with the values of growth-performance parameters, liver weight, renal ZEA accumulation, total protein content, transaminase activity at 42 days, and antioxidant indexes being similar to those in the control group. These results suggested that MPal supplementation could promote growth performance, attenuate liver damage, and improve the antioxidant abilities of broilers fed ZEA-contaminated diet by reducing ZEA accumulation.

Key Words—Broilers, Kidney, Liver, Modified Palygorskite, Zearalenone.

INTRODUCTION

Zearalenone (ZEA) is a secondary metabolite produced naturally by numerous species of *Fusarium* fungi (D'Mello *et al*., 1999; Hestbjerg *et al*., 2002; Glenn, 2007) and is detected in many cereal crops including corn and wheat; ZEA occurs more readily when crops are stored in high-humidity environments (Cavret and Lecoeur, 2006; Binder *et al*., 2007). ZEA has strong estrogenic and anabolic activities (Turcotte *et al*., 2005; Zinedine *et al*., 2007) and adverse effects on genital organs and reproductive performance are found widely in chickens (Allen *et al*., 1981a), pigs (Jiang *et al*., 2010; Wang *et al*., 2010), turkeys (Allen *et al*., 1983), and rats (Lemke *et al*., 2001; Denli *et al*., 2017). Aside from reproductive toxicity, ZEA has the ability to penetrate into the various organs (Buranatragool *et al*. 2015), resulting in incremental ZEA accumulation in tissue (Mirocha *et al*., 1982; Wang *et al*., 2013). Liver plays an essential role in ZEA metabolism (Malekinejad *et al*., 2006; Koraichi *et al*., 2012), and can be damaged easily by ZEA; greater hepatic ZEA accumulation has been observed (Wang *et al*., 2013) than in their origins, suggesting that liver is one of the main target organs of ZEA. Studies conducted on animals have demonstrated that ZEA can cause liver lesions as shown by the abnormality of serum biochemical parameters related to hepatic function (Abbès *et al*., 2006; Šperanda *et al*., 2006; Jia *et al*., 2014). Kidneys have extensive blood flow and a variety of transporters due to their important role in excretion, reabsorption, and general homeostasis, which could lead to the delivery of large amounts of ZEA to kidneys and increased renal ZEA residue (Ouanes *et al*., 2003; Abbès *et al*., 2006; Jia *et al*., 2014); this indicates that kidneys are another important

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target organ of ZEA. In addition, oxidative stress has been suggested as a possible result of the toxicity of ZEA. Evidence of oxidative damage induced by ZEA, including lipid peroxidation and imbalanced antioxidant enzymes, was observed in both *in vivo* (Jiang *et al*., 2011; Jiang *et al*., 2012) and *in vitro* studies (Abidessefi *et al*., 2004, 2008; Othmen *et al*., 2008; Ben *et al*., 2015).

The contamination of grains and feed with mycotoxins including ZEA is a permanent challenge in animal nutrition (Zinedine *et al*., 2007). One of the more encouraging approaches to eliminating or attenuating the harmful effects of mycotoxins on animals is the supplementation of adsorptive materials in the diet. The most commonly used mycotoxin adsorbents are clay minerals such as sodium calcium aluminosilicate (Che *et al*., 2011; Yiannikouris *et al*., 2013), montmorillonite (Daković *et al*., 2008, 2012), and zeolite (Daković *et al*., 2007; Trailović *et al*., 2013). Due to their hydrophilic, negatively charged surfaces, most of the raw mineral adsorbents are effective at adsorbing polar toxin molecules such as aflatoxins but are less effective at adsorbing nonpolar or less polar toxin molecules such as ZEA (Bočarovstančić *et al*., 2011). *In vitro* and *in vivo* studies have demonstrated that modified minerals (*e.g.* zeolite and montmorillonite) may increase mineral-surface hydrophobicity, providing greater adsorptive affinity for ZEA than their natural analogs (Daković *et al*., 2007; Feng *et al*., 2008; Jiang *et al*., 2012). Palygorskite (Pal) is a hydrated magnesium-rich silicate clay mineral with nanorod-like crystal morphology and nano-channels (Bergaya and Lagaly, 2013). Pal has been used widely as a non-toxic, eco-friendly, economic, and efficient adsorbent in animal feed in both academia and in the feed industry (Tian *et al*., 2015; Wang *et al*., 2015). Unfortunately, raw Pal has poor adsorption capacity for ZEA because of its structure (Schell *et al*., 1993). Modification of Pal by means of physical and/or chemical methods to generate greater affinity for ZEA is necessary, therefore. The purpose of the present study was to modify raw Pal with quaternized chito-oligosaccharides, and to use the modified Pal (MPal) as a ZEA-targeted adsorbent which could adsorb ZEA efficiently *in vitro* and to evaluate the protective efficiency of MPal with respect to broilers fed the ZEA-contaminated diet by determining growth performance, hepatonephric weight, ZEA residue, serum metabolites, and antioxidant capacities.

MATERIALS AND METHODS

Materials

Raw Pal was provided by Jiangsu Sinitic Biotech Co., Ltd. (Xuyi, Jiangsu, P.R. China). The chemical compositions of raw Pal determined using a Minipal 4 X-ray fluorescence spectrometer (PANalytical, Almelo, The Netherlands) are as follows: 522.9 g/kg SiO_2, 122.9 g/kg Al_2O_3, 56.7 g/kg MgO, 86.5 g/kg Fe_2O_3, 25.9 g/kg CaO, 23.8 g/kg K_2O, and 1.8 g/kg Na_2O. Chito-oligosaccharide (food grade), with the molecular formula $(C_6H_{11}NO_4)_n$ and average molecular weight of 1701 a.m.u., was obtained from Jinan Haidebei Marine Bioengineering Co., Ltd. (Shandong, P.R. China). Glycidyltrimethylammonium chloride (GTAC) was purchased from Sigma-Aldrich Co. (St. Louis, Missouri, USA). All other reagents were of analytical grade and solutions were prepared with deionized water (the resistivity of which was 18.2 MOhm-cm).

Preparation of MPal

Firstly, chito-oligosaccharide was modified with GTAC to prepare its quaternary ammonium derivative N-(2-hydroxy) propyl-3-trimethyl ammonium chito-oligosaccharide chloride (HTACO), using acetic acid as the catalyst, following the method of Seong *et al*. (2000). Secondly, the raw Pal was modified with HTACO according to the following procedure: 1000 g of the Pal was dispersed uniformly in 2 mass% of the aqueous solution of sulfuric acid at the solid/liquid ratio of 1:5 by mechanical stirring for 2 h. Then, 150 g of the HTACO was added to the suspension and the mixture was stirred continuously for 4 h at 40ºC. Finally, the solid product was separated by centrifugation, dried at 100ºC for 4 h, and ground with a DFY-500 Universal Stainless Steel High-Speed Pulverizer (Shanghai LeiGu Instrument Co., Ltd., Shanghai, P.R. China) and passed through a 200-mesh sieve. The product was marked as 'MPal'.

Characterization of Pal and MPal

The structures and morphologies of Pal and MPal were characterized by X-ray diffraction (XRD) and transmission electron microscopic (TEM) analyses, and the results are shown in Figure 1. The XRD patterns were collected using an X'Pert PRO X-ray powder diffractometer (PANalytical Co., Almelo, The Netherlands) equipped with a CuKα radiation source (λ = 0.1541 nm; 40 kV, 40 mA) from 3 to 70º2θ at a scanning rate of 8.34º2θ/min, with a step interval of ~0.167º2θ, divergence slit of 0.5º, and anti-scatter slit of 1º. The TEM images were collected using a JEM-1200 EX/S transmission electron microscope (JEOL, Tokyo, Japan) at an accelerating voltage of 200 kV. The characteristic diffraction peaks (Figure 1) of the Pal phase at 8.32º2θ (110 crystal plane), 13.64º2θ (200 crystal plane), 16.52º2θ (130 crystal plane), 19.80º2θ (040 crystal plane), and 27.98º2θ (400 crystal plane) were observed in the XRD patterns of both raw Pal and MPal, and the reflection peaks of quartz at 26.7º2θ and 20.9º2θ also appeared in the XRD patterns, indicating that Pal and quartz crystals were present in the MPal samples. The modification of Pal did not affect its crystal structure, which suggests that the modifier was attached mainly to the surface of the Pal. The rod crystals could be seen clearly in the TEM images of Pal

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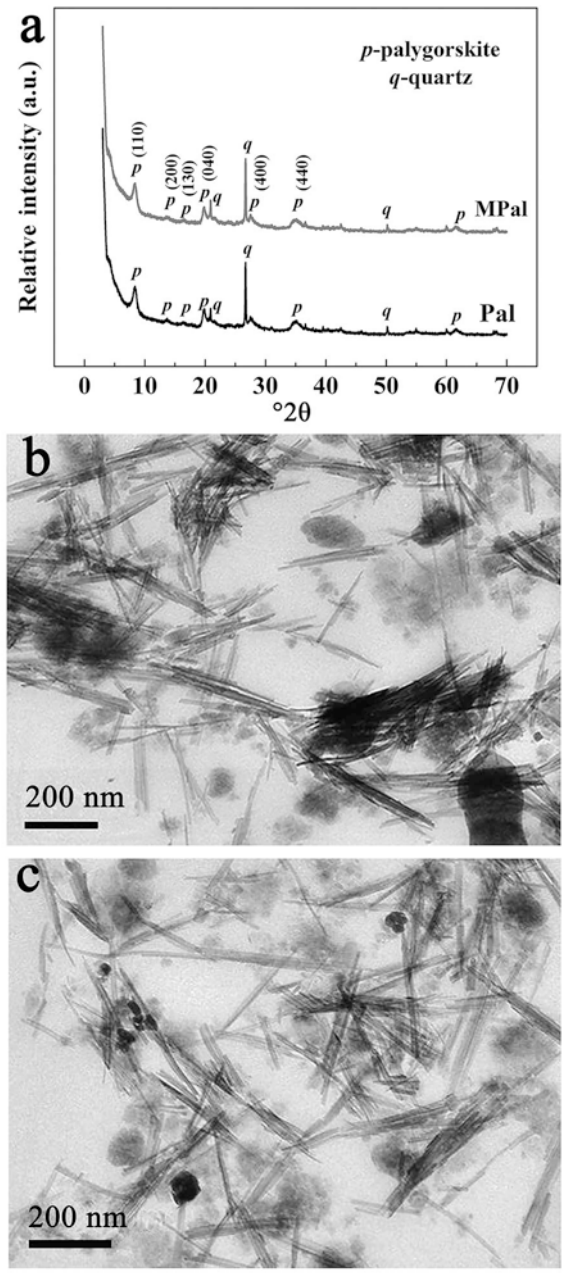

Figure 1. (a) XRD patterns of Pal and MPal samples; (b) TEM image of Pal; and (c) TEM image of MPal.

and MPal (Figure 1b, c), and the degree of dispersion of the rod crystals improved following modification under the action of mechanical stirring.

Collection of ZEA-contaminated corn

Before the experiment, corn samples were collected and the ZEA content in each sample determined; a corn sample with a ZEA content of 927.27 μg/kg, which exceeded the maximum limit set by the Hygienic Standard for Feeds of China (AQSIQ, 2017) was selected as the ZEA-contaminated diet preparation for the present study.

Experimental design, animals, and husbandry

The experimental design and all procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee. One hundred and forty-four one-day-old, healthy Arbor Acres broilers (50% of which were male), with similar initial body weight, were obtained from a commercial hatchery and were assigned randomly to groups to undergo one of three dietary treatments with six replicates of eight birds each. The three groups included: (1) a basal diet with normal corn (control group); (2) a ZEA-contaminated diet (ZEA group); and (3) the contaminated diet supplemented with 1 g/kg MPal (MPal-ZEA group). The basal diet was formulated in accordance with the recommendation of the National Research Council (NRC, 1994). The contaminated diet was prepared by using the naturally ZEA-contaminated corn to replace normal corn in the control diet. Representative samples of each experimental diet were taken for nutrient- and mycotoxin-content analysis before feeding commenced (Table 1). Concentrations of aflatoxin B_1 (AFB_1), deoxynivalenol (DON), and ZEA were tested by enzyme-linked immunosorbent assay (R-Biopharm AG, Darmstadt, Germany).

All broiler chicks were placed in wired cages (60 cm long × 190 cm wide × 50 cm tall), and housed in a temperature-controlled room with continuous lighting. The temperature was maintained at 32−34ºC for the first three days, and then decreased by 2−3ºC per week to a final temperature of 20ºC. The birds were allowed free access to water and mash feed. At 21 and 42 days of age, the broilers were weighed in replicate after a 12 h period of feed deprivation and feed intake was recorded in replicate to calculate average daily gain (ADG), average daily feed intake (ADFI), and feed:gain ratio (F:G). Birds that died during the experimental period were weighed and the data were included for the F:G calculation.

Sample collection and processing

At 21 and 42 days, one female bird close to the average weight was selected from each replicate and weighed after feed deprivation for 12 h. Blood samples of ~5 mL each were collected from a wing vein and centrifuged at 4000 × *g* for 15 min at 4ºC to separate the serum which was frozen at −20ºC until further analysis. The broilers were euthanized by cervical dislocation after blood sampling. The liver and kidney were removed quickly and weighed to calculate the relative organ weight, and then stored immediately at −20ºC for subsequent analysis. Relative organ weights were expressed on a relative body weight basis (g/kg).

Determination of ZEA residue

The ZEA residue in the liver and kidney was determined using an enzyme-linked immunosorbent kit

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Table 1. Compositions and nutrient levels (%), and mycotoxin concentrations (μg/kg) in the experimental diets.

Items[1]	—— Starter diet (1−21 days) ——			—— Finisher diet (22−42 days) ——		
	Control group	ZEA group	MPal-ZEA group	Control group	ZEA group	MPal-ZEA group
Ingredients						
Corn	57.00			62.00		
Contaminated corn		57.00	57.00		62.00	62.00
Soybean meal	32.60	32.60	32.60	28.00	28.00	28.00
Corn gluten meal	3.00	3.00	3.00	2.00	2.00	2.00
Soybean oil	3.00	3.00	3.00	4.00	4.00	4.00
Limestone	1.23	1.23	1.23	1.30	1.30	1.30
Diacalcium phosphate	2.00	2.00	2.00	1.60	1.60	1.60
L-Lysine	0.32	0.32	0.32	0.31	0.31	0.31
DL-Methionine	0.15	0.15	0.15	0.11	0.11	0.11
Premix[1]	0.40	0.40	0.40	0.38	0.38	0.38
Sodium choride	0.30	0.30	0.30	0.30	0.30	0.30
MPal[2]			0.10			0.10
Nutrient levels						
Metabolizable energy (Kcal/g)	3.00	3.00	3.00	3.10	3.10	3.10
Crude protein	21.55	21.55	21.55	19.33	19.33	19.33
L-Lysine	1.22	1.22	1.22	1.10	1.10	1.10
Methionine	0.50	0.50	0.50	0.43	0.43	0.43
Calcium	1.01	1.01	1.01	0.93	0.93	0.93
Available phosphorus	0.46	0.46	0.46	0.39	0.39	0.39
Methionine + cysteine	0.86	0.86	0.86	0.76	0.76	0.76
Analyzed nutrient levels						
Gross energy (cal/g)	3848	3852	3846	4057	4011	4018
Crude protein	21.34	21.14	20.71	19.73	19.20	18.91
Calcium	1.26	1.23	1.22	1.12	1.05	1.05
Total phosphorus	0.65	0.67	0.65	0.59	0.60	0.57
Mycotoxin concentrations						
Aflatoxin B_1	0.59	0.66	0.56	0.68	0.65	0.68
Deoxynivalenol	716.65	2528.81	2645.63	540.03	2844.50	2820.77
Zearalenone	14.08	512.82	507.84	19.38	597.22	555.87

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU (international units); vitamin D_3 (cholecalciferol), 3000 IU; vitamin E (all-rac-α-tocopherol), 30 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 600 mg; calcium pantothenate, 10 mg; pyridoxine·HCl, 4 mg; biotin, 0.04 mg; folic acid, 1 mg; vitamin B_{12} (cobalamin), 0.013 mg; Fe (from ferrous sulphate), 80 mg; Cu (from copper sulfate), 8.0 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

(R-Biopharm AG, Darmstadt, Germany) according to the manufacturer's protocol.

Serum metabolites

The concentrations of total protein (TP) and albumin (ALB) in serum were determined using commercial kits (Shanghai Kehua Bioengineering Co., Ltd., Shanghai, P.R. China) with an automatic biochemical analyzer (Olympus, Tokyo, Japan). The activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were analyzed spectrophotometrically using commercial diagnostic kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China). One unit (U) of AST (or ALT) is defined as the pyruvic acid produced over 1 min at 25ºC which would decrease the absorbance by 0.001 by oxidizing the reduced nicotinamide adenine dinucleotide to form the oxidized nicotinamide adenine dinucleotide at 340 nm.

Assay of antioxidant parameters in the liver and kidney

Upon analysis, ~0.3 g of liver and kidney samples were homogenized with ice-cold saline buffer (0.85%, pH = 7.4) at a ratio of 1:9 (w/v) using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, Ohio, USA), and then centrifuged at $4000 \times g$ for 15 min at 4ºC. The supernatant was obtained and stored at −20ºC for subsequent determination of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities, and the concentrations of glutathione (GSH) and mal-

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Table 2. Growth performance of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal.

Items[1,2]	Control group	ZEA group	MPal-ZEA group	SEM[3]	*P* value
1−21 days					
ADG (g/day)	39.02	39.45	39.58	0.90	0.967
ADFI (g/day)	60.65	60.54	62.05	0.98	0.802
F:G (g/g)	1.56	1.54	1.57	0.02	0.779
21−42 days					
ADG (g/day)	89.51[a]	82.39[b]	88.36[a]	1.12	0.011
ADFI (g/day)	160.82[a]	150.00[b]	161.52[a]	2.04	0.024
F:G (g/g)	1.80	1.82	1.83	0.01	0.467
1−42 days					
ADG (g/day)	68.48	64.50	68.04	0.82	0.088
ADFI (g/day)	118.40	112.22	119.41	1.38	0.061
F:G (g/g)	1.73	1.74	1.76	0.01	0.332

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] ADG, average daily gain; ADFI, average daily feed intake; F:G, feed/gain ratio.
[3] SEM, standard error of the mean.
[a,b] Means within a column with different superscripts differ significantly (P <0.05).

ondialdehyde (MDA). The related antioxidant parameters were measured using the clinical chemistry assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China). The activities of T-SOD and GSH-Px were expressed as U per milliliter for serum or U per milligram of protein for tissues, and the GSH and MDA contents were expressed as nanomoles per milliliter for serum or nanomoles per milligram of protein for tissues. One U of T-SOD was calculated as the amount of enzyme per milligram of protein of tissues (or per milliliter of serum) that would result in 50% inhibition of the rate of nitrite generation at 37ºC. One U of GSH-Px activity was calculated as the amount of enzyme per milligram of protein of tissues (or per milliliter of serum) that would catalyze the conversion of 1 μmol/L of reduced glutathione to oxidized glutathione at 37ºC in 5 min. Total protein concentration in the liver and kidney was determined according to the method of Bradford (1976) using a protein assay kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, P.R. China).

Statistical analysis

All data were subjected to one-way analysis of variance (ANOVA) using *SPSS* 21.0 statistical software (SPSS Inc., Chicago, Illinois, USA). The significance of differences between various treatments was tested using Duncan's multiple range tests. The P values of <0.05 were considered significant.

RESULTS

Growth performance

Compared with the control group, feeding the ZEA-contaminated diet reduced the ADG and ADFI of broilers during the finisher period (P <0.05, Table 2), which were promoted with the supplementation of MPal to levels comparable with those in the control group (P <0.05).

Relative weights of liver and kidney

Relative hepatic weight in the ZEA group was less at 21 days but greater at 42 days than in the control group (P <0.05, Table 3), and it was normalized by MPal supplementation (P <0.05). Relative renal weight, however, remained unchanged for all three treatments (P >0.05).

ZEA residue in the liver and kidney

Broilers ingesting the ZEA-contaminated diet exhibited increases in ZEA accumulations in the liver and kidney at

Table 3. Relative organ weights of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal (g/kg).

Items[1]	Control group	ZEA group	MPalZEA group	SEM[2]	*P* value
21 days					
Liver	26.30[a]	23.42[b]	26.49[a]	0.56	0.032
Kidney	14.38	13.87	13.98	0.27	0.742
42 days					
Liver	18.28[b]	20.97[a]	19.04[b]	0.41	0.010
Kidney	12.32	11.32	11.36	0.34	0.420

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] SEM, standard error of the mean.
[a, b] Means within a column with different superscripts differ significantly (P <0.05).

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Table 4. ZEA residue level in the liver and kidney of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal.

Items[1]	Control group	ZEA group	MPal-ZEA group	SEM[2]	*P* value
21 days					
Liver (μg/kg)	0.57[c]	2.50[a]	1.81[b]	0.23	<0.001
Kidney (ng/kg)	76.11[b]	355.33[a]	78.52[b]	37.88	<0.001
42 days					
Liver (μg/kg)	0.84[c]	4.60[a]	2.76[b]	0.40	<0.001
Kidney (ng/kg)	156.14[b]	1307.41[a]	342.16[b]	140.24	<0.001

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] SEM, standard error of the mean.
[a, b] Means within a column with different superscripts differ significantly (P <0.05).

both 21 and 42 days when compared with the control group (P <0.05, Table 4), which were decreased with the inclusion of MPal (P <0.05), with the value of renal ZEA residue being similar to those in the control group (P >0.05).

Serum metabolites

Compared with the control group, the ZEA-contaminated diet reduced TP content at 21 and 42 days and ALB content at 21 days, but increased ALT activity at 21 and 42 days and AST activity at 42 days (P <0.05, Table 5). The supplementation of MPal increased the TP content at 21 and 42 days but decreased AST and ALT activities at 42 days to normal levels compared with the control group (P <0.05). The ALT activity at 21 days in the MPal-ZEA group was also intermediate amongst the three treatments (P <0.05).

Antioxidant capacities

Broilers given the ZEA-contaminated diet had increased T-SOD activity in the liver at 21 and 42 days, decreased T-SOD activity in the kidney at 21 and 42 days and in the serum at 42 days, increased the MDA accumulation in the liver and kidney at 42 days, and reduced GSH content in the kidney at 21 days, when compared with those of birds fed the basal diet (P <0.05, Tables 6, 7). The values of the aforementioned parameters were normalized (except T-SOD activity in the liver at 21 days) by the supplementation of MPal (P <0.05).

DISCUSSION

In the three diets deployed, the AFB_1 and DON levels were significantly less than the maximum limit set by National Standards of China (AQSIQ, 2017). The ZEA content in the contaminated diet exceeded the maximum limit of National Standard by AQSIQ (2017) and was ~35 times greater than that in the control group. The harmful consequences of feeding the contaminated diet to broilers were due to ZEA-induced effects, therefore.

Table 5. Serum biochemical parameters of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal.

Items[1, 2]	Control group	ZEA group	MPal-ZEA group	SEM[3]	*P* value
21 days					
TP (g/L)	37.19[a]	32.43[b]	36.84[a]	0.81	0.023
ALB (g/L)	15.63[a]	13.04[b]	13.93[a,b]	0.42	0.025
AST (U/L)	4.46	4.87	4.70	0.17	0.650
ALT (U/L)	18.59[c]	22.34[a]	20.72[b]	0.48	<0.001
42 days					
TP (g/L)	49.17[a]	40.55[b]	46.36[a]	1.18	0.004
ALB (g/L)	15.61	16.21	15.94	0.23	0.598
AST (U/L)	4.86[b]	6.47[a]	4.88[b]	0.20	<0.001
ALT (U/L)	19.61[b]	25.21[a]	18.02[b]	0.94	<0.001

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] TP, total protein; ALB, albumin; AST, aspartate aminotransferase; ALT, alanine aminotransferase.
[3] SEM, standard error of the mean.
[a, b] Means within a column with different superscripts differ significantly (P <0.05).

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Table 6. Antioxidant capacities in the liver, kidney, and serum of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal at 21 days of age.

Items[1, 2]	Control group	ZEA group	MPal-ZEA group	SEM[3]	*P* value
Liver					
T-SOD (U/mg protein)	224.48[b]	253.15[a]	234.57[a,b]	4.98	0.049
GSH (mg/g protein)	0.75	0.85	0.88	0.05	0.520
GSH-Px (U/mg protein)	21.79	20.98	21.36	0.27	0.211
MDA (nmol/mg protein)	0.67	0.61	0.64	0.03	0.714
Kidney					
T-SOD (U/mg protein)	257.38[a]	224.08[b]	225.70[b]	5.84	0.020
GSH (mg/g protein)	0.72[a]	0.55[b]	0.68[a]	0.03	0.021
GSH-Px (U/mg protein)	16.61	16.69	16.14	0.92	0.581
MDA (nmol/mg protein)	0.77	0.82	0.92	0.03	0.078
Serum					
T-SOD (U/mL)	108.88	115.35	105.65	2.33	0.216
GSH (mg/L)	2.10	2.19	2.10	0.10	0.921
GSH-Px (U/mL)	794.44	776.46	800.02	14.98	0.508
MDA (nmol/mL)	4.40	3.71	3.77	0.14	0.099

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] T-SOD, total superoxide dismutase; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.
[3] SEM, standard error of the mean.
[a, b] Means within a column with different superscripts differ significantly (P <0.05).

Growth performance

The effects of ZEA on the growth performance of animals have been reported previously; the results have been inconsistent, however. In the present study, reduced ADG and ADFI of broilers fed the ZEA-contaminated diet were observed during the finisher period, which was consistent with findings by Nesic *et al.* (2005) who discovered that ZEA compromised the growth performance of pigs. Previous studies have demonstrated that feeding *Fusarium*-contaminated grains could impair

Table 7. Antioxidant capacities in the liver, kidney, and serum of broilers fed the basal diet, the ZEA-contaminated diet, or the ZEA-contaminated diet supplemented with MPal at 42 days of age.

Items[1, 2]	Control group	ZEA group	MPal-ZEA group	SEM[3]	*P* value
Liver					
T-SOD (U/mg protein)	140.78[b]	195.77[a]	137.65[b]	8.52	0.003
GSH (mg/g protein)	0.98	0.89	0.87	0.03	0.234
GSH-Px (U/mg protein)	19.96	20.21	20.06	0.23	0.315
MDA (nmol/mg protein)	0.98[b]	1.49[a]	1.12[b]	0.08	0.019
Kidney					
T-SOD (U/mg protein)	236.65[a]	185.34[b]	212.66[a]	6.77	0.002
GSH (mg/g protein)	1.08	1.00	0.94	0.04	0.416
GSH-Px (U/mg protein)	13.87	13.57	13.66	0.63	0.627
MDA (nmol/mg protein)	1.01[b]	1.42[a]	1.10[b]	0.05	0.001
Serum					
T-SOD (U/mL)	128.82[a]	108.22[b]	125.86[a]	3.07	0.003
GSH (mg/L)	2.38	2.36	2.24	0.08	0.786
GSH-Px (U/mL)	875.25	899.67	833.03	16.04	0.115
MDA (nmol/mL)	5.09	5.87	5.29	0.16	0.109

[1] Control group, ZEA group, and MPal-ZEA group; broilers were given the basal diet, the ZEA-contaminated diet, and the ZEA-contaminated diet supplemented with MPal.
[2] T-SOD, total superoxide dismutase; GSH, glutathione; GSH-Px, glutathione peroxidase; MDA, malondialdehyde.
[3] SEM, standard error of the mean.
[a, b] Means within a column with different superscripts differ significantly (P <0.05).

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growth performance in broilers (Swamy *et al*., 2002a), hens (Dänicke *et al*., 2002; Yegani *et al*., 2006), and turkeys (Girish *et al*., 2005). No significant adverse consequences for animal-growth performance from pure ZEA were observed by Jiang *et al*. (2011) and Allen *et al*. (1981b). This discrepancy could be related to species, management, or dosages and types of mycotoxins. The supplementation of MPal increased the ADG and ADFI of broilers during the finisher period in the present study. The findings have demonstrated that MPal inclusion could reduce ZEA residue in the liver and kidney. The improved growth performance of broilers resulting from MPal supplementation in this study would, therefore, be attributed to the strong adsorptive capacity of MPal to ZEA. Previous studies have proved that Pal supplementation could improve growth performance in broilers (Zhang *et al*., 2017) and hens (Chalvatzi *et al*., 2014), which would have contributed to growth promotion in the present study also.

Hepatonephric weight and ZEA residue

Relative organ weight is usually used to reflect the growth and development of the organ in question. ZEA toxicity is manifested in the reproductive tract, liver, and kidney, and hepatonephric weight could, therefore, be considered as a parameter to reflect the toxicity of ZEA. In the present study, relative liver weight was decreased at 21 days whereas it increased at 42 days in the ZEA group compared with the control group, suggesting that feeding the ZEA-contaminated diet could disrupt normal development of liver. Similar results were observed by Aravind *et al*. (2003) and Li *et al*. (2012) who reported that broilers ingesting a diet contaminated with ZEA exhibited abnormal relative weight of liver. The liver and kidney are the main target organs for ZEA toxicity, and ZEA contamination could result in ZEA accumulation in the liver and kidney. Feeding a regime of 2 mg/kg ZEA could increase the ZEA residue in the liver and muscle of pigs according to Wang *et al*. (2013). Early investigations reported that feeding diets contaminated with ZEA could lead to ZEA residue in the liver (Mirocha *et al*., 1982) and increased ZEA accumulation in edible tissue (Dänicke *et al*., 2003). Consistent with the above-mentioned findings, increased ZEA residue in the liver and kidney of broilers fed the ZEA-contaminated diet was observed in the present study. Interestingly, relative kidney weight remained unaltered by the ZEA-contaminated diet, and was consistent with results reported previously by Aravind *et al*. (2003) and Dänicke *et al*. (2003), which may be the result of smaller ZEA residue in the kidney than that in the liver. The variations, in terms of degree of organ damage and ZEA residue level in the tissues (kidney and liver) observed in the present study, indicated that liver is more sensitive to ZEA than kidney. Supplementation of MPal normalized the relative liver weight and reduced ZEA accumulation in the both liver and kidney. *In vitro* studies (Feng *et al*., 2008; Jiang *et al*., 2012) demonstrated that other modified mineral adsorbents (*e.g*. montmorillonite and zeolite) exhibited a large adsorption capacity for ZEA. Significant decreases in ZEA accumulation in the liver and kidney after MPal supplementation found in the present study would, therefore, result from the large adsorption capacity of MPal for ZEA in the gastrointestinal tract, and normalized liver weight may, therefore, be associated closely with reduced ZEA residue in the liver.

Serum metabolites

Serum transaminases such as ALT and AST usually exist in the cytoplasm of hepatocyte and are used commonly as a reliable index to evaluate the degree of damage to the tissue or to the whole body (Homolka, 1969). Transaminase would be released from hepatocytes into the blood when liver is damaged (Čonková *et al*., 2001). In the present study, the ZEA-contaminated diet increased significantly the ALT and AST activities in the serum, suggesting that feeding a ZEA-contaminated diet could cause liver damage, in agreement with previous studies about broilers (Swamy *et al*., 2002b; Jiang *et al*., 2014), pigs (Jiang *et al*., 2012; Wang *et al*., 2012), and mice (Abbès *et al*., 2006). Serum TP and ALB are produced by liver, the levels of which are usually used to reflect protein synthesis capacity of liver. Broilers fed the ZEA-contaminated diet had reduced TP and ALB concentrations. Similar results were found by Chowdhury and Smith (2007) who observed that plasma TP and ALB concentrations decreased when birds ingested grains contaminated naturally with ZEA. Metabolic disorder of liver occurred in parallel with simultaneously increased hepatic ZEA residue, suggesting that damaged liver function could be associated with increased ZEA accumulation. In pigs, modified montmorillonite supplementation was shown by Jiang *et al*. (2012) to reduce transaminase activity in the serum. Similarly, the inclusion of MPal decreased ALT and AST activities in the serum of broilers ingesting the ZEA-contaminated diet in the present study. The normalized levels of serum metabolites in broilers fed the ZEA-contaminated diet after MPal supplementation were similar to simultaneously reduced hepatic ZEA residue, and this could be attributed to the adsorption capacity of MPal for ZEA.

Antioxidant capacities

The organism maintains a dynamic balance between the oxidative and antioxidant systems under normal physiological conditions (Sies, 1991) and this balance might be broken down by adverse factors, leading to accumulation of free radicals and cellular lipid peroxidation. MDA is the end product of lipid peroxidation and its accumulation could be seen as a vital indicator for lipid peroxidation (Vaca *et al*., 1988). GSH is a major antioxidant in the cell to scavenge free radicals

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and helps to regenerate other antioxidants (Meister, 1994). T-SOD and GSH-Px play important roles in the antioxidant enzyme defense system that can catalyze the dismutation of superoxide anions and the decomposition of reactive oxygen species, respectively (Kramer *et al*., 1988; Zelko *et al*., 2002). Extensive *in vitro* studies have proved that ZEA could increase MDA accumulation (Abidessefi *et al*., 2004; Kouadio *et al*., 2005; Ferrer *et al*., 2009) but deplete GSH content (Hassen *et al*., 2007) in several types of cells. Likewise, *in vivo* experiments have also demonstrated that animals treated with diverse levels of ZEA exhibited greater MDA concentrations, smaller GSH contents, and disrupted antioxidant enzyme activities (T-SOD and GSH-Px) in the various tissues and/or serum (Zourgui *et al*., 2008; Marin *et al*., 2013; Jia *et al*., 2014; Liu *et al*., 2014). In agreement with the results above, feeding the ZEA-contaminated diet to broilers resulted in increased MDA accumulation, decreased GSH content, and disrupted T-SOD activities in this study. The imbalanced redox system caused by the ZEA-contaminated diet suggested that oxidative stress could be a possible pathway of ZEA toxicity to broilers. Abnormal antioxidant indexes paralleled the simultaneously increased ZEA residue in the current study, indicating that compromised redox status would be associated closely with increased ZEA accumulation. In the present study, broilers fed the ZEA-contaminated diet supplemented with MPal exhibited reduced MDA concentration, increased GSH content, and balanced T-SOD activity. This result was consistent with the findings by Jiang *et al*. (2012) who reported that modified montmorillonite could partially ameliorate ZEA-induced adverse effects on the antioxidant capacities. Dietary MPal supplementation could reduce ZEA accumulation in both the liver and kidney, and the protective effects in terms of the antioxidant capacities of broilers fed the ZEA-contaminated diet would, therefore, be due to its adsorptive capacity for ZEA.

CONCLUSIONS

Feeding the ZEA-contaminated diet retarded growth performance, increased hepatonephric ZEA residue, disordered liver weight and serum metabolites, and induced oxidative damage in broilers, and most of these adverse consequences were attenuated by the supplementation of MPal. The results suggested the possibility of application of MPal to ameliorate the detrimental effects on broilers from ZEA.

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