Livestock Science 210 (2018) 15–20

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Effects of modified montmorillonite adsorbent on performance, egg quality, serum biochemistry, oxidation status, and immune response of laying hens in late production

X.Y. Qu[a,b,*], J.F. Chen[a,b], C.Q. He[a,b], F. Chi[c], S.L. Johnston[c]

[a] College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China
[b] Hunan Co-Innovation Center of Animal Production Safety, Changsha, Hunan, China
[c] Amlan International, Chicago, IL, United States

ARTICLE INFO

Keywords:
Laying hens
Immune response
Modified montmorillonite
Mycotoxin
Oxidation status
Performance

ABSTRACT

This study was conducted to investigate the effects of dietary supplementation with calcium montmorillonite (Ca-MMT) on layers' oxidation status, immune response, serum biochemistry, performance, and egg quality. A total of four hundred-eighty 75-wk-old laying hens were randomly assigned to 5 treatments with 8 replicates per treatment and 12 hens in each replicate. The hens were fed the basal diet supplemented with 0, 0.3, 0.6, 0.9, or 1.2 g Ca-MMT/kg for 70 d. The Ca-MMT supplementation linearly or quadratically ($P < 0.05$) increased yolk index, serum total superoxide dismutase activity, and interleukin-2 and immunoglobulin G concentrations at d 35, and shell thickness, and serum and liver glutathione peroxidase activity at d 70. With increasing supplementation of Ca-MMT in the basal diet, serum activity of alkaline phosphatase decreased both linearly and quadratically ($P < 0.05$) at d 35, and alanine aminotransfer as activity and tumor necrosis factor-alpha concentration decreased linearly or quadratically ($P < 0.05$) at d 70. The evidence indicates that increasing Ca-MMT concentration improved yolk index and shell thickness, and partially enhanced hens' antioxidant capability and immune function.

1. Introduction

Both bacterial and fungal toxins have a significant effect on poultry production. Overgrowth of bacteria, such as *Escherichia coli* and *Salmonella*, in the intestinal tract causes induced tissue oxidative stress and excessive immune response (Li et al., 2015; Shao et al., 2013). This frequently results in decreased productivity, contaminated poultry products, and even increased mortality (Cravens et al., 2015; Jing et al., 2014). Mycotoxins are toxic secondary metabolites produced by several fungi species that grow and reproduce on raw feed materials or compound feeds (Gregorio et al., 2014). Many studies have demonstrated the adverse effects of mycotoxins on the growth or productive performance, antioxidant status, and immune system of broiler chickens (Diaz et al., 2016; Liu et al., 2016), ducks (Han et al., 2008), quails (Ogido et al., 2004), and laying hens (Denli et al., 2008; Rizzi et al., 2003). Therefore, it is important to alleviate the detrimental effect of bacteriotoxins and mycotoxins on poultry production.

Montmorillonite (MMT), one of the aluminosilicate mineral clays, has a large surface area (there are numerous micropores), good adsorb, and ion exchange capacity, and thus it can be used to adsorb toxins (Murray, 2000). An in vitro study demonstrated that MMT could adsorb *Escherichia coli* and *Staphylococcus aureus* (Hu et al., 2002). It has been reported that MMT can treat intestinal infections caused by *Escherichia coli* and *Salmonella* (Herrera et al., 2000). In addition, the MMT could mitigate the mycotoxin-induced adverse effects on poultry growth performance, oxidation status, and immune functions (Ghareeb et al., 2013; Manafi, 2012; Osselaere et al., 2013). Modified MMT has greater interlayer spacing, more micropores and pore volume, and therefore stronger adsorptive power. The modified MMT, used in the present study is a calcium montmorillonite (Ca-MMT) with improved hydrophobic binding capability, has been shown to bind intestinal bacterial and fungal toxins, and improve broiler and pig performance (Cravens et al., 2015; Jiang et al., 2012). Furthermore, previous studies in our laboratory have demonstrated that Ca-MMT can effectively ameliorate the detrimental effects of low concentrations of mycotoxins on laying hens at peak production, resulting in improved egg production (Chen et al., 2016). Our assumption is that Ca-MMT may improve productive performance of laying hens during later phases of the laying cycle, and thus prolong the egg production period. Therefore, this study was conducted to investigate the effects of dietary supplementation with Ca-

* Corresponding author at: College of Animal Science and Technology, Hunan Agricultural University, Changsha, Hunan, China.
E-mail address: quxy99@126.com (X.Y. Qu).

https://doi.org/10.1016/j.livsci.2018.01.021

MMT on layers' performance, egg quality, serum biochemistry, oxidation status, and immune response during late production.

2. Materials and methods

2.1. Adsorbent

The Ca-MMT (Calibrin®-Z; Amlan International, Chicago, IL, US), used in the current study was a thermally processed mineral adsorbent. Its main components are: calcium montmorillonite > 70%, amorphous hydrated silicon dioxide > 15%, and other mineral elements < 15%.

2.2. Experimental design, diets, and hen management

A total of 480 75-wk-old laying hens (Lohmann Brown; Tianxin Breeding of Yellow Broilers Liability Co. Ltd, Changsha, Hunan, China) were randomly assigned to 5 treatments with 8 replicates per treatment and 12 hens per replicate. Three hens were raised in each cage with 4 cages per replicate. The experimental diets included basal diet supplemented with 0, 0.3, 0.6, 0.9, and 1.2 g Ca-MMT/kg. The basal diet was a corn-soybean meal based and formulated in accordance with the established guidelines (NY/T 33–2004; Feeding standard of chicken, Ministry of Agriculture of the People's Republic of China, Beijing, Wen et al., 2004) to meet the nutrient requirements of laying hens. Diet composition is shown in Table 1. The experimental diets were packed in covered containers and stored in a dry and well-ventilated storeroom.

All the procedures were approved by the Institutional Animal Care and Use Committee of the Hunan Agricultural University. Hens were housed in an environmentally controlled room. During wk 1–5 of the experiment, the temperature and relative humidity in the room were 20.64 ± 1.76 °C and 68.90 ± 0.90%, respectively. During wk 6–10 of the experiment, the temperature and relative humidity in the room were 22.60 ± 1.04 °C and 74.46 ± 2.74%, respectively. The hens were exposed to a 16-h photo-period for a period of 10 wk. Before the trial started the hens were allowed a period of 7 d to adapt to their individual cages, and all hens were fed the basal diet during that time. All hens were then fed the assigned experimental diets for 70 d. Feed and water were available for hens to consume ad libitum.

Table 1
Composition of the basal diet (as-fed basis)[a].

Item	Amount
Ingredient (%)	
Corn	64.00
Soybean meal	22.00
Limestone	9.00
Premix[b]	5.00
Total	100.00
Calculated composition	
ME (MJ/kg)	11.32
CP (%)	15.58
Lys (%)	0.78
Met + Cys (%)	0.56
Ca (%)	3.93
Available P (%)	.36

[a] ME = metabolizable energy; and CP = crude protein.

[b] Premix provided per kilogram of diet: vitamin A, 6000 IU; vitamin D_3, 2500 IU; vitamin B_1, 1.75 mg; vitamin B_2, 5.5 mg; vitamin B_6, 4 mg; vitamin B_{12}, 0.18 mg; vitamin E, 25 mg; vitamin K_3, 2.25 mg; biotin, 0.14 mg; folic acid, 0.8 mg; nicotinic acid, 34 mg; pantothenic acid (Ca pantothenate), 12 mg; phytase, 400 U; chloride, 350 mg; Fe (ferrous sulfate), 75 mg; Cu (copper sulfate), 7.5 mg; Zn (zinc sulfate), 60 mg; Mn (manganese sulfate), 60 mg; I (potassium iodide), 1.25 mg; Se (sodium selenite), 0.15 mg; Ca, 9.5 g; P, 2.0 g; and NaCl, 3.7 g.

2.3. Analysis of mycotoxins

Diets were sampled and mycotoxins determined by the Asia Mycotoxin Analysis Center (Chaoyang University of Technology, Taichung, Taiwan) as described by Jiang et al. (2010). Aflatoxins (AFL, 10–23 μg/kg), deoxynivalenol (715–1258 μg/kg) zearalenone (ZEA, 156–185 μg/kg), ochratoxin A (0–1 μg/kg), and fumonisins (1013 to 1809 μg/kg) were observed in the basal diet at d 1, d 35, and d 70. The detection limit for mycotoxins was 1 μg/kg for AFL, 4.5 μg/kg for deoxynivalenol and ZEA, 2.5 μg/kg for T-2 toxin, 1 μg/kg for ochratoxin A, and 3.5 μg/kg for fumonisins.

2.4. Performance and egg quality

Egg production and egg weight were recorded daily by replicate, and feed consumption was recorded weekly by replicate to calculate egg mass, egg production, feed intake, and feed conversion ratio. Egg quality was measured on 4 eggs collected randomly from each replicate at d 35 and d 70. These eggs were kept at 4 °C and analyzed within 2 d after collection to determine egg quality index. Egg length, egg width, yolk width, and yolk height were measured by using an electronic digital caliper (SH14100025; Shenhan, Shanghai, China). The egg shape index was calculated by dividing egg length by egg width, and the yolk index was calculated by dividing yolk height by yolk width. Yolk color and Haugh unit were measured by using an egg analyzer (EA-01; Orka Food Technology Ltd, Ramat HaSharon, Tel Aviv-Yafo, Israel). Shell thickness was determined by using a digital micrometer (NFN380; FHK, Bunkyo-ku, Tokyo, Japan) and shell strength was measured by using an egg force reader (EFR-01; Orka Food Technology Ltd).

2.5. Serum biochemistry, oxidation status, and immunoglobulin and cytokine concentrations

At d 35 and d 70, one hen was randomly selected from each replicate. Blood samples (about 7 mL/hen) were obtained from the wing vein using a disposable lancet, then immediately transferred into a non-heparinized tube. Within 2 h, the serum was obtained by centrifuging the blood at 2500 × *g* at 4 °C for 10 min and stored at − 80 °C until further analysis. Concentrations of total protein, total cholesterol, calcium, and glucose, and activities of alkaline phosphatase (ALP) and alanine aminotransferase (ALT) in serum were measured by using an Mindray automatic analyzer (BS-300; Shenzhen Mindray Bio-Medical Electronics Co., Ltd, Shenzhen, Guangdong, China) according to the commercial kits (Shenzhen Mindray Bio-Medical Electronics Co., Ltd). Activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px), and concentrations of malondialdehyde (MDA) and total antioxidant capacity (T-AOC) were determined using an assay kit (A001–1-1; A005; A003-1; A015-1; Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) with Microplate reader (Multiskan GO; Thermo Fisher Scientific, Waltham, CT, US) according to manufacturer's guideline. Concentrations of interleukin-2 (IL-2), tumor necrosis factor-alpha (TNF-α), immunoglobulin (Ig) A, IgG, and IgM were measured by ELISA kit (CSB-E06755Ch; CSB-E11231Ch; CSB-E11232Ch; CSB-E09872Ch; CSB-E16200C; Cusabio Biotech Co., Ltd, Wuhan, Hubei, China) according to manufacturer's instructions.

2.6. Liver oxidation status

At the end of the experiment, one hen was randomly selected from each replicate. Those hens were killed by cervical dislocation and bleeding. Their livers were removed, packed, and frozen until analyzed for liver oxidation status. Upon analysis, liver samples were thawed, rinsed with ice-cold deionized water, and dried with filter paper. The samples were then homogenized (T10 BS25; IKA, Baden-Württemberg, Germany) at 4 °C for 2 min in a 12 mL centrifuge tube with ice-cold saline according at a ratio of 1:9 (W/V) then centrifuged at 1500 × *g* at