Dietary palygorskite-based antibacterial agent supplementation as an alternative to antibiotic improves growth performance, intestinal mucosal barrier function, and immunity in broiler chickens

Pingping Zha,* Yueping Chen,* Shiqi Wang,* Aiqin Wang ,† and Yanmin Zhou*,[1]

*College of Animal Science and Technology, Nanjing Agricultural University, Nanjing, 210095, People's Republic of China; and †Key Laboratory of Clay Mineral Applied Research of Gansu Province, Center of Eco-material and Green Chemistry, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, 730000, People's Republic of China

ABSTRACT The aim of this study was to evaluate effects of palygorskite-based antibacterial agent (**PAA**) as an alternative to antibiotic on growth performance, intestinal barrier function, and immunity in broilers. Three hundred and eighty-four mixed-sex 1-day-old Ross 308 broiler chicks were allocated into 6 groups of 8 replicates with 8 birds each. Birds were given a basal diet, an antibiotic diet (50 mg/kg chlortetracycline), and the basal diet supplemented with 250, 500, 1,000, and 2,000 mg/kg PAA for 42 d, respectively. Compared with control group, supplementing 1,000 mg/kg PAA reduced overall feed conversion ratio ($P < 0.05$), with its value being similar to that of antibiotic group ($P > 0.05$). However, a higher level of PAA (2,000 mg/kg) increased feed conversion ratio during the late period ($P < 0.05$). The 1,000 and 2,000 mg/kg PAA decreased plasma endotoxin and D-lactate levels at 42 d ($P < 0.05$) to comparable values ($P > 0.05$). The 1,000 mg/kg PAA decreased jejunal crypt depth, while 500 and 1,000 mg/kg PAA increased the ratio between jejunal villus height and crypt depth at 42 d ($P < 0.05$), with their values being similar to antibiotic group ($P > 0.05$). The highest level of PAA increased 42-d jejunal mucosal secretory immunoglobulin A and immunoglobulin M concentrations ($P < 0.05$). The 1,000 and 2,000 mg/kg PAA reduced 21-d interleukin-1β and tumor necrosis factor-α (**TNF-α**) levels in serum and ileal mucosa and 42-d interferon-γ level in serum and jejunal mucosa ($P < 0.05$), which did not differ from antibiotic group ($P > 0.05$). Moreover, PAA administration, regardless of its dosage, reduced 42-d serum TNF-α concentration, and 500 to 2,000 mg/kg PAA decreased 21-d and 42-d jejunal and 42-d ileal mucosal TNF-α levels ($P < 0.05$), with their values being comparable with antibiotic group ($P > 0.05$). The results suggested that PAA as an alternative to antibiotic could improve growth performance, intestinal barrier function, and immunity of broilers, and its optimal dosage was 1,000 mg/kg.

Key words: palygorskite-based antibacterial agent, growth performance, intestinal barrier, immunity, broilers

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INTRODUCTION

The antibiotics have been practiced globally in animal production for decades since its first discovery as the growth promoters in mid-1940s (Castanon, 2007). Although results are variable, available literature has generally shown that the incorporation of antibiotics to livestock and poultry feed could promote growth, improve feed conversion efficiency, save feeding cost, reduce morality, and prevent or cure clinical and subclinical infections and diseases (Dibner and Richards, 2005; Durso and Cook, 2014; Adhikari et al., 2020). However, the usage of antibiotics in animal feed has drawn increasing public concern due to their accumulation in animal-derived food (Vishnuraj et al., 2016; Bacanlı and Başaran, 2019), threat to environment (Gothwal and Shashidhar, 2015), and contribution to the occurrence, development, and prevalence of antibacterial resistance in pathogenic bacteria (Ferri et al., 2017). These harmful consequences would eventually result in health problems and even death in humans through food chain and environmental exposure (Verraes et al., 2013; Radhouani et al., 2014; Huijbers et al., 2015). The Chinese government has forbidden the usage of antibiotics as the drug feed additives

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[1]Corresponding author: zhouym6308@163.com

to promote growth of poultry, swine, ruminant, and aquatic animals since July 1, 2020 as a result of the detrimental side effects of antibiotics. The phasing out of antibiotic growth promoters from animal feeds, however, has led to poor growth performance, intestinal health problems, high incidence of enteric and systemic diseases, and high mortality, eventually causing considerable economic loss (Cervantes, 2015). A meta-analysis study has concluded that broilers fed antibiotic-free diets exhibited a lower weight gain and a higher feed conversion ratio than those fed antibiotic growth promoters, and the withdrawal of antibiotics caused an economic loss of 0.03 USD per broiler (Maria Cardinal et al., 2019). It has, therefore, become necessary to develop antibiotic alternatives to maintain animal health and to improve overall production as well as to ensure food safety.

Palygorskite (**Pal**), also known as attapulgite, is a natural crystalline hydrated magnesium aluminum silicate mineral with a fibrous morphology, and it contains a continuous two-dimensional tetrahedral sheet but lacks continuous octahedral sheets (Galán, 1996). The presence of micropores and channels along with fibrous structure and fine particle size in this clay mineral allow its wide application in agriculture, industry, environment, and biomedicine, including as carriers, absorbents, fertilizers, adhesives, anti-caking agents, and pharmaceuticals (Galán, 1996; Murray, 2000). Aside from these applications, the Pal has been incorporated in monogastric and ruminant animal feeds, either as the feed additive or raw feed ingredient. Up to now, most of available studies regarding the usage of Pal in different animal species is the original Pal itself rather than its modified counterparts actually. Available literature in poultry and swine has concluded that dietary supplementation with the original Pal could improve physical quality of pelleted feed, growth performance, nutrient digestibility, immunity, antioxidant status, and intestinal mucosal barrier function and integrity of animals (Pappas et al., 2010; Zhang et al., 2013; Lv et al., 2015; Chen et al., 2016a,b; Zhang et al., 2017), and exhibit an anti-inflammatory effects in a rodent model of 12-O-tetradecanoylphorbol-13-acetate-induced inflammation (Juárez et al., 2016; López-Pacheco et al., 2017) and in broiler chickens subject to lipopolysaccharide-induced immunological stress (Chen et al., 2020b) by inhibiting production of proinflammatory cytokines and altering the infiltration of different leukocyte cells to an inflammation site.

The characteristics of high surface area, high porosity, and good ion-exchange capacity in clay minerals (e.g., montmorillonite, zeolite, and Pal) would allow their subsequent modifications through physical and chemical reactions to improve their biological activities when feeding to animals (Murray, 2000; Liu et al., 2021). Previous studies have demonstrated that metal ion-modified clay mineral (montmorillonite) could effectively improve growth performance, intestinal barrier function, antioxidant capacity, and gut microbiota composition in weaned piglets (Hu et al., 2012a,b; Song et al., 2013; Jiao et al., 2015, 2018; Li et al., 2021). Moreover, zeolite- and montmorillonite-based antibacterial agents have been found to possess the potential to replace antibiotic in weaned piglets and broiler chicks, and their supplementation could improve growth performance and intestinal health (Ke et al., 2014; Tang et al., 2014b). Likewise, the modification of raw Pal could also improve its bioactivity. Our previous studies have shown that the bioavailability of Pal-based zinc source (zinc-loaded Pal) was higher than zinc sulfate alone (Yan et al., 2016) and its administration could improve growth performance, meat quality, and oxidative status in broilers (Yang et al., 2016, 2017). The antimicrobial activity of raw Pal is extremely poor and even absent, but in vitro studies have demonstrated that the antibacterial agent prepared by the direct modification of Pal by essential oil or zinc oxide have been reported to exhibit favorable antibacterial activities against pathogenic bacteria, and was even better than the corresponding essential oil (Lei et al., 2017; Hui et al., 2020a), which, in turn, suggested that Pal-based antibacterial agent (**PAA**) has a promising potential to replace antibiotic growth promoter. However, little is known about the potential of PAA as an alternative to antibiotics used in broiler production actually. In this study, we therefore, supplemented different levels of PAA to broiler feed, and then evaluated the possibility of PAA to replace antibiotic by determining their growth performance, intestinal mucosal barrier function, and immunity-related parameters in a 42-d feeding experiment. The finding of this study would provide a new reference for the development of antibiotic alternatives and the maintenance of growth performance and health in broiler chickens fed antibiotic-free diets.

MATERIALS AND METHODS

The Preparation of PAA

The Pal used in the current study was acquired from Huangnishan Mine (Xuyi County, Jiangsu Province, P. R. China). The chemical composition of Pal was: SiO_2, 523 g/kg; Al_2O_3, 123 g/kg; Fe_2O_3, 86.5 g/kg; MgO, 56.7 g/kg; CaO, 26.0 g/kg; K_2O, 23.8 g/kg; Na_2O, 1.80 g/kg. Prior to use, the raw Pal was purified according to the method described previously (Chen et al., 2016a). Briefly, the raw Pal mineral was rolled on a 3-roller machine for one time, and then dispersed in water at solid/liquid ratio 1/10. The aqueous dispersion was passed through a 300-mesh sieve to remove the large grains of quartz or aggregates. The solid was separated from the dispersion by filter-pressing process, and then dried and smashed as powder (particle size <75 μm) for further use.

The PAA was synthesized using the purified Pal as a carrier and was kindly provided by Jiangsu Sinitic Biological Technology Co, Ltd., P. R. China. The compositions of this PAA were quaternary ammonium chitooligosaccharides (**QACOS**)/ZnO/Pal nanocomposite and essential oil/Pal nanocomposite, but their

specific proportions were unavailable due to commercial sensitivity. The QACOS/ZnO/Pal nanocomposite was synthesized by incorporating QACOS onto the ZnO/Pal; the antibacterial capacity of this compound was superior than QACOS and ZnO/Pal, and its minimum inhibitory concentration for *Escherichia coli* and *Staphylococcus aureus* was 0.5 mg/mL and 1 mg/L, respectively (Hui et al., 2020b). The essential oil/Pal nanocomposite mainly consisting of thymol/Pal and carvone/Pal was prepared by hybridizing essential oil with Pal through mechanical grinding, and the minimum inhibitory concentration of these nanocomposites for *Escherichia coli* and *Staphylococcus aureus* ranged from 2.0 to 4.0 mg/mL (Zhong et al., 2021).

Animals, Diets, and Treatments

All animal experiments in this study were performed in strict accordance with a protocol approved by the Nanjing Agricultural University Animal Care and Use Committee and conformed to guidelines on laboratory animal use set by Jiangsu Provincial Department of Science and Technology, P.R. China (SYXK (SU) 2017-0007).

A total of 384 mixed-sex one-day-old Ross 308 broiler chicks with similar initial body weight (46.26 ± 0.27 g) were allocated into one of the 6 groups in a completely randomized design for a 42-d feeding experiment. Each experimental group consisted of 8 replicates (pen) of 8 birds each (4 males and 4 females). Birds were fed an antibiotic-free basal diet (Control group), an antibiotic diet, and the basal diet supplemented with 250, 500, 1,000, and 2,000 mg/kg PAA, respectively. The antibiotic diet was prepared by supplementing the basal diet with 50 mg/kg chlortetracycline of diet (Jinhe Biotechnology Co, Ltd., Inner Mongolia province, P.R. China). The ingredient composition and nutrient levels of the basal diet are shown in Table 1. Mash feed and water were provided to the broiler chickens ad libitum throughout this experiment. Birds were reared in steel cages with plastic floors (120 cm × 60 cm × 50 cm) in a temperature- and light-controlled room and were subjected a light-dark cycle of 23 h of light and 1 h of dark during the entire experimental period. The temperature in the chicken house was maintained between 32°C and 34°C during the initial 3 d, and then decreased by 2°C to 3°C each consecutive week until it reached 24°C. The daily mean relative humidity was maintained at approximately 70% during the first 3 d and at 60 to 65% thereafter.

Table 1. Composition and nutrient level of the basal diet (%, as-fed basis unless otherwise stated).

Items	1 to 21 d	22 to 42 d
Ingredients		
Corn	57.00	62.00
Soybean meal	32.60	28.00
Corn gluten meal	3.00	2.00
Soybean oil	3.00	4.00
Limestone	1.23	1.30
Dicalcium phosphate	2.00	1.60
L-Lysine	0.32	0.30
DL-Methionine	0.15	0.10
Sodium chloride	0.30	0.30
Premix[1]	0.40	0.40
Calculated nutrient levels		
Apparent metabolizable energy (MJ/kg)	12.55	12.98
Crude protein	21.55	19.33
Calcium	1.01	0.93
Available phosphorus	0.46	0.39
Lysine	1.22	1.10
Methionine	0.50	0.43
Methionine + cystine	0.90	0.72

[1]Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3,000 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 sulfate), 80 mg; Cu (from copper sulphate), 8.0 mg; Mn (from manganese sulphate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; Se (from sodium selenite), 0.3 mg.

Sample Collection

At 21 and 42 d of age, all birds were weighed individually at early morning (around 5:30 a.m.) after a 12-h feed deprivation (water remained available during feed deprivation). After that, one male bird with a body weight nearest to the pen average from each replicate (8 birds from each experimental group and 48 birds in total) was selected and sampled at around evening (around 4:30 p.m.) in order to avoid starvation-induced intestinal barrier damage. The whole blood sampled from the wing vein via puncture were collected into both clean glass tubes coated with heparin and clean plastic centrifuge tubes without anticoagulant, and kept refrigerated until separation. Serum was separated after centrifugation at 4,450 × *g* for 15 min at 4°C, while plasma was harvested after centrifugation at 2,000 × *g* for 15 min at 4°C. The serum and plasma samples were then immediately frozen at −20°C until further analysis. Birds were euthanized by cervical dislocation after blood sampling and necropsy was performed. The thymus, spleen, and bursa of Fabricius were collected from the euthanized birds and weighed to determine the relative organ weight according to the following formula: relative organ weight (g/kg) = organ weight/terminal body weight. After measurement of immune organ weight, the jejunum (from the end of the duodenum to the Meckel's diverticulum) and ileum (from Meckel's diverticulum to the ileocecal junction) were removed free from mesentery and connective tissues and placed on a chilled stainless steel tray immediately. The mid-jejunum and mid-ileum segments were then excised, flushed gently with ice-cold phosphate-buffered saline (pH = 7.4), and immediately immersed in 10% fresh chilled neutral-buffered formalin solution for histological evaluation. The remaining jejunum and ileum were dissected longitudinally and washed with ice-cold phosphate-buffered saline to remove residual digesta. The jejunal and ileal mucosa were then scraped using a sterile glass microscope slide and collected into sterile frozen

tubes, which were immediately snap-frozen in liquid nitrogen and then stored at liquid nitrogen tank for subsequent analysis.

Growth Performance Determination

Birds were weighed on a pen basis at 21 and 42 d of age after a 12-h feed withdrawal period, and the feed consumption of broiler chickens was recorded on pen basis to determine average daily feed intake (**ADFI**), average daily gain (**ADG**), and feed conversion ratio (**FCR**) during the early (1−21 d), late (22−42 d), and overall (1−42 d) experimental periods, respectively. The weight of mortalities and culled birds from each pen were used to adjust FCR.

Measurement of Plasma D-Lactate and Endotoxin

The plasma D-lactate concentration was measured by an enzyme-linked immunosorbent assay kit (cat. no. H263, Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, P.R. China), strictly following the manufacturer's instructions. The quantitative chromogenic tachypleus amebocyte lysate for endotoxin (pyrogen) detection kit was employed in the determination of plasma endotoxin level, and all measurements were done according to the instructions of manufacturer (Xiamen Bioendo Technology Co., Ltd., Xiamen, P.R. China).

Determination of Immunoglobulins and Cytokines

The homogenate was prepared in sodium chloride solution prior to the determination of intestinal mucosal concentrations of immunoglobulins and cytokines. Briefly, approximately 0.3 g of intestinal mucosal samples were homogenized with a chilled 154 mmol/L sterile sodium chloride solution at a ratio of 1: 9 (wt/vol), using a PRO-PK-02200D homogenizer (Pro Scientific, Inc., Monroe, CT). The acquired homogenized solution was then centrifuged at 4,450 × g for 15 min at 4°C. The resulting supernatant was collected in 7 aliquots and were immediately stored at −20°C until they were analyzed.

The concentrations of secretory immunoglobulin A (**sIgA**, cat. no.H108-2), immunoglobulin M (**IgM**, cat. no.H109), and immunoglobulin G (**IgG**, cat. no.H106) in the jejunal and ileal mucosa were quantified using corresponding colorimetric assay kits (Nanjing Jiancheng Bioengineering Institute), according to the manufacturer's instructions. Chicken-specific enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute) were employed for the determination of interferon-γ (**IFN-γ**, cat. no.H025), interleukin-1β (**IL-1β**, cat. no. H002), and tumor necrosis factor-α (**TNF-α**, cat. no. H052) concentrations in both serum and intestinal mucosa (jejunal and ileal mucosa) of broiler chickens, following the manufacturer's protocols. The inter- and intra-assay variation coefficients of enzyme-linked immunosorbent assay kits were less than 8 and 10%, respectively. All results of intestinal mucosa were adjusted against corresponding total protein concentration in each sample for intersample comparison. The intestinal mucosal total protein levels were measured according to Bradford method, using crystalline bovine serum albumin as a standard protein (Sigma-Aldrich, St Louis, MO).

Histological Measurement

After a 24-h fixation in buffered formalin, the intestinal segments were transferred to 70% ethanol. Formalin-fixed intestinal segments were then dehydrated in ethanol series, cleared in xylene, and embedded in paraffin blocks, which were cooled before sectioning. A 5-μm serial paraffin section was prepared, and the slides were then deparaffinized with xylene, rehydrated through graded alcohols, and stained with hematoxylin and eosin. Photographs of the stained slides were pictured with a microscope and digitalised using a Nikon ECLIPSE 80i light microscope equipped with a computer-assisted morphometric system (Nikon Corporation, Tokyo, Japan). Villus height and crypt depth of 10 well-preserved villi and crypts in each slide were measured.

Statistical Analysis

Data were analyzed by one-way analysis of variance (**ANOVA**) using SPSS statistical software (Ver.22.0 for windows, SPSS Inc., Chicago, IL). The statistical model for data analysis was: $Y_{ij} = \mu + T_i + \varepsilon_{ij}$, where Y_{ij} = dependent observation; μ = overall mean; T_i = effect of treatment; ε_{ij} = the random error. A pen (replicate) was the experimental unit for the growth performance data, while an individual bird from each pen was the experimental unit for other measured parameters, including immune organ weight, intestinal morphology, and the concentrations of immunoglobulins and cytokines. Orthogonal polynomial contrasts were also employed to test the linear and quadratic effects of the increasing levels of PAA. Differences among treatments were tested using Duncan's multiple range test. The differences were considered as statistically significant when $P < 0.05$, and results were presented as means with their pooled standard errors.

RESULTS

Growth Performance

Dietary PAA administration quadratically reduced FCR of broiler chickens during the late (22−42 d of age) and overall (1−42 d of age) experimental periods (Table 2, $P < 0.05$). Compared with the control group, antibiotic supplementation decreased FCR of broilers during the late and entire periods ($P < 0.05$). In

Table 2. Effects of dietary palygorskite-based antibacterial agent supplementation on the growth performance of broiler chickens.

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1,2]	CON	ANT	250	500	1,000	2,000	SEM[3]	ANOVA	Linear	Quadratic
1−21 d										
ADG (g/d)	33.9	35.0	34.9	34.7	34.9	35.3	0.4	0.968	0.220	0.985
ADFI (g/d)	49.2	50.4	51.3	50.2	50.0	50.9	0.6	0.934	0.383	0.811
FCR (g: g)	1.46	1.44	1.47	1.45	1.43	1.44	0.01	0.581	0.169	0.448
22−42 d										
ADG (g/d)	79.7	82.8	80.4	80.2	82.9	75.2	1.0	0.208	0.961	0.169
ADFI (g/d)	149.8	148.4	150.2	148.6	150.2	146.1	1.4	0.964	0.771	0.836
FCR (g: g)	1.88[b]	1.80[c]	1.87[b]	1.86[bc]	1.82[bc]	1.94[a]	0.01	<0.001	0.578	0.019
1−42 d										
ADG (g/d)	56.8	58.9	57.6	57.4	58.9	55.2	0.5	0.341	0.551	0.266
ADFI (g/d)	98.6	98.7	100.0	98.5	99.2	97.6	0.8	0.974	0.592	0.757
FCR (g: g)	1.74[ab]	1.68[c]	1.73[ab]	1.72[ab]	1.69[c]	1.77[a]	0.01	0.001	0.817	0.044

[a,b,c]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, aontrol group; ANT, antibiotic group.
[2]Abbreviations: ADG, average daily gain; ADFI, average daily feed intake; FCR, feed conversion ratio.
[3]SEM = Standard error of the mean (n = 8).

contrast, supplementing 1,000 mg/kg PAA reduced FCR of birds during the overall period when compared with the control group ($P < 0.05$), with its value being similar to that of antibiotic group ($P > 0.05$). Moreover, the overall FCR of birds (1−42 d) receiving a basal diet supplemented with 1,000 mg/kg PAA was significantly lower than other three PAA-treated groups ($P < 0.05$). However, birds fed a basal diet supplemented with 2,000 mg/kg PAA had the highest value of FCR during the late period in comparison with the control, antibiotic, and other PAA-supplemented groups ($P < 0.05$). Likewise, the overall FCR of birds in 2,000 mg/kg PAA-supplemented group was higher than their counterparts receiving a basal diet supplemented with antibiotic or 1,000 mg/kg PAA ($P < 0.05$). There were no significant differences in ADG and ADFI during all experimental periods (early, late, and overall experimental periods) as well as FCR during the early grower period among treatments ($P > 0.05$).

Relative Immune Organ Weight

Feeding a basal diet supplemented PAA linearly increased relative weight of bursa of Fabricius in broiler chickens at 21 d of age (Table 3, $P < 0.05$). However, neither antibiotic nor PAA administration altered the relative weights of spleen (21 and 42 d), thymus (21 and 42 d), or bursa of Fabricius (42 d; $P > 0.05$).

Circulating Endotoxin and D-lactate Levels

Dietary PAA administration linearly reduced plasma endotoxin level at 42 d and D-lactate level at both 21 and 42 d in broiler chickens (Table 4, $P < 0.05$). Compared with the control group, dietary administration with 1,000 and 2,000 mg/kg PAA reduced plasma endotoxin and D-lactate levels at 42 d of age ($P < 0.05$), and there were no significant differences between these PAA-treated groups ($P > 0.05$). However, dietary antibiotic supplementation did not affect circulating endotoxin or D-lactate level when compared with the control group ($P > 0.05$).

Intestinal Morphology

Dietary administration with antibiotic or PAA did not alter intestinal morphology of broiler chickens at 21 d of age (Table 5, $P > 0.05$). The supplementation of PAA to a basal diet linearly decreased crypt depth and

Table 3. Effects of dietary palygorskite-based antibacterial agent supplementation on the relative immune organ weight of broiler chickens (g/kg).

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1]	CON	ANT	250	500	1,000	2,000	SEM[2]	ANOVA	Linear	Quadratic
21 d										
Spleen	0.92	0.90	0.93	0.93	0.87	0.75	0.03	0.624	0.196	0.273
Bursa of Fabricius	1.64	1.94	2.04	2.22	2.30	2.32	0.08	0.152	0.016	0.490
Thymus	4.23	5.35	5.08	4.65	5.41	5.55	0.16	0.107	0.160	0.523
42 d										
Spleen	5.77	3.73	4.19	4.52	4.67	4.42	0.24	0.222	0.164	0.254
Bursa of Fabricius	3.90	4.10	4.44	4.52	4.79	5.00	0.23	0.772	0.158	0.952
Thymus	11.36	6.92	10.93	9.06	10.08	8.35	0.48	0.064	0.911	0.240

[1]CON, control group; ANT, antibiotic group.
[2]SEM = Standard error of the mean (n = 8).

Table 4. Effects of dietary palygorskite-based antibacterial agent supplementation on the plasma endotoxin and D-lactate levels of broiler chickens.

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1]	CON	ANT	250	500	1,000	2,000	SEM[2]	ANOVA	Linear	Quadratic
21 d										
Endotoxin (EU/mL)	0.64	0.62	0.62	0.52	0.48	0.47	0.03	0.453	0.075	0.864
D-lactate (mmol/L)	0.68	0.52	0.53	0.50	0.47	0.42	0.03	0.107	0.007	0.612
42 d										
Endotoxin (EU/mL)	0.65[a]	0.58[a]	0.54[ab]	0.45[abc]	0.31[bc]	0.28[c]	0.04	0.010	0.001	0.797
D-lactate (mmol/L)	0.63[a]	0.55[a]	0.52[ab]	0.54[a]	0.42[bc]	0.38[c]	0.19	0.001	<0.001	0.520

[a,b,c]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, control group; ANT, antibiotic group.
[2]SEM = Standard error of the mean (n = 8).

linearly increased the ratio between villus height and crypt depth in both jejunum and ileum of broiler chickens at 42 d of age ($P < 0.05$). Compared with the control group, dietary antibiotic administration reduced jejunal villus height and crypt depth of broiler chickens at 42 d of age ($P < 0.05$). However, dietary PAA supplementation, irrespective of its supplemental level, did not alter 42-d jejunal villus height of birds when compared with control group ($P > 0.05$), and the value of this parameter in the four PAA-supplemented groups was all greater than that of antibiotic group ($P < 0.05$). Compared with the control group, the administration with 1,000 mg/kg PAA reduced jejunal crypt depth of broilers at 42 d of age ($P < 0.05$), and this parameter in 1,000 mg/kg PAA-supplemented group did not differ from antibiotic and other PAA-treated groups ($P > 0.05$). Supplementing 500 and 1,000 mg/kg PAA increased the ratio between villus height and crypt depth in the jejunum of broilers at 42 d of age ($P < 0.05$), and the value of this index in these groups was both comparable with antibiotic group ($P > 0.05$). However, the highest level of PAA (2,000 mg/kg) reduced the ratio of jejunal villus height to crypt depth of birds at 42 d of age when compared with the control group ($P < 0.05$). Unlike jejunum, dietary administration with 2,000 mg/kg PAA increased the ratio of villus height to crypt depth in the ileum of birds at 42 d in comparison with control and antibiotic groups ($P < 0.05$), and there was no significant difference among those PAA-supplemented groups ($P > 0.05$).

Intestinal Mucosal Immunoglobulins

Neither antibiotic nor PAA administration affected intestinal mucosal sIgA, IgM, or IgG concentration (jejunum and ileum) of broiler chickens at 21 d of age (Table 6, $P > 0.05$). Dietary PAA supplementation linearly increased jejunal mucosal sIgA and IgM concentrations ($P < 0.05$), quadratically increased jejunal mucosal

Table 5. Effects of dietary palygorskite-based antibacterial agent supplementation on the small intestinal morphology of broiler chickens.

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1,2]	CON	ANT	250	500	1,000	2,000	SEM[3]	ANOVA	Linear	Quadratic
21 d										
Jejunum										
VH (μm)	1,263.63	1,128.06	1,268.09	1,304.81	1,285.76	1,290.37	25.47	0.380	0.719	0.894
CD (μm)	240.24	229.1	253.27	239.56	242.56	231.31	7.35	0.956	0.695	0.542
VH: CD (μm: μm)	5.64	5.23	5.17	5.65	5.62	5.64	0.21	0.966	0.865	0.681
Ileum										
VH (μm)	913.07	905.51	861.95	756.76	801.89	792.98	24.56	0.343	0.092	0.476
CD (μm)	202.92	181.68	200.35	201.51	175.21	172.58	5.48	0.370	0.060	0.373
VH: CD (μm: μm)	4.77	5.21	4.45	3.8	4.75	4.71	0.19	0.426	0.961	0.206
42 d										
Jejunum										
VH (μm)	1,385.17[a]	904.15[b]	1,461.09[a]	1,461.21[a]	1,386.24[a]	1,454.60[a]	34.91	<0.001	0.601	0.550
CD (μm)	246.38[a]	161.98[c]	225.05[ab]	225.13[ab]	191.83[bc]	207.16[ab]	6.59	0.002	0.018	0.769
VH: CD (μm: μm)	5.75[b]	6.73[ab]	6.75[abc]	7.25[a]	7.03[a]	5.63[c]	0.16	0.004	0.005	0.283
Ileum										
VH (μm)	893.41	912.77	912.77	895.46	956.72	997.49	19.61	0.640	0.221	0.734
CD (μm)	213.05	196.02	196.02	174.68	182.2	172.74	6.57	0.255	0.034	0.612
VH: CD (μm: μm)	4.57[b]	4.76[b]	4.76[b]	5.16[ab]	5.32[ab]	5.80[a]	0.14	0.038	0.003	0.888

[a,b,c]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, control group; ANT, antibiotic group.
[2]Abbreviations: CD, crypt depth; VH, villus height; VH: CD, villus height/crypt depth.
[3]SEM = Standard error of the mean (n = 8).

Table 6. Effects of dietary palygorskite-based antibacterial agent supplementation on the concentrations of intestinal mucosal immunoglobulins in broiler chickens (μg/mg protein).

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1,2]	CON	ANT	250	500	1,000	2,000	SEM[3]	ANOVA	Linear	Quadratic
21 d										
Jejunum										
sIgA	1.13	1.18	1.13	1.05	1.31	1.18	0.05	0.820	0.529	0.761
IgM	3.23	3.23	2.99	2.94	3.51	3.10	0.07	0.198	0.549	0.250
IgG	33.92	36.56	32.49	28.27	41.99	34.14	1.91	0.461	0.581	0.608
Ileum										
sIgA	1.38	1.38	1.33	1.25	1.38	1.24	0.04	0.876	0.528	0.992
IgM	3.06	3.07	2.86	3.22	3.14	3.10	0.08	0.883	0.603	0.863
IgG	39.50	39.78	34.50	32.99	42.96	40.09	1.24	0.171	0.503	0.100
42 d										
Jejunum										
sIgA	1.23[b]	1.23[b]	1.22[b]	1.46[ab]	1.47[ab]	1.53[a]	0.04	0.035	0.006	0.692
IgM	3.30[b]	3.18[b]	3.43[ab]	3.11[b]	3.63[ab]	3.87[a]	0.08	0.026	0.022	0.070
IgG	39.75[ab]	38.59[ab]	36.27[b]	33.72[b]	44.91[ab]	48.69[a]	1.55	0.048	0.052	0.018
Ileum										
sIgA	1.53	1.43	1.43	1.33	1.45	1.63	0.03	0.144	0.573	0.014
IgM	3.19	3.20	2.87	3.28	3.66	3.33	0.09	0.305	0.230	0.595
IgG	40.86	41.50	37.27	36.18	42.54	49.97	1.84	0.328	0.037	0.005

[a,b,c]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, control group; ANT, antibiotic group.
[2]Abbreviations: IgM, immunoglobulin M; IgG, immunoglobulin G; sIgA, secretory immunoglobulin A.
[3]SEM = standard error of the mean (n = 8).

IgG and ileal mucosal sIgA concentrations ($P < 0.05$), and linearly and quadratically increased ileal mucosal IgG concentration ($P < 0.05$) of broilers at 42 d of age. Compared with the control and antibiotic groups, the supplementation of 2,000 mg/kg PAA increased jejunal mucosal sIgA and IgM concentrations of broilers at 42 d of age ($P < 0.05$). Moreover, broilers fed a basal diet supplemented with 2,000 mg/kg PAA has the highest level of jejunal mucosal IgG at 42 d among treatments ($P < 0.05$).

Serum and Intestinal Mucosal Cytokines

Dietary PAA supplementation (Table 7) linearly reduced the concentrations of serum IL-1β and TNF-α at 21 d ($P < 0.05$) and the concentrations of serum IFN-γ, IL-1β, and TNF-α at 42 d ($P < 0.05$), respectively. Compared with the control group, antibiotic supplementation reduced the concentrations of serum IL-1β and TNF-α at 21 d ($P < 0.05$) and the concentrations of serum IFN-γ and TNF-α at 42 d ($P < 0.05$), respectively. The supplementation of PAA at the levels of 1,000 and 2,000 mg/kg reduced the concentrations of serum IL-1β and TNF-α at 21 d and the serum IFN-γ concentration at 42 d when compared with control group ($P < 0.05$), and the values of these aforementioned parameters in these PAA-supplemented groups did not differ from antibiotic group ($P > 0.05$). The administration of PAA, regardless of its level, reduced serum TNF-α concentration at 42 d in comparison with the control group ($P < 0.05$), with its lowest value being observed in 2,000 mg/kg PAA-supplemented group. Moreover, the serum TNF-α concentration of birds at 42 d in the four PAA-supplemented groups was comparable with that of antibiotic group ($P > 0.05$).

The supplementation of PAA linearly decreased jejunal mucosal IFN-γ, IL-1β, and TNF-α concentrations at 21 d, ileal mucosal IL-1β and TNF-α concentrations at both 21 and 42 d, and jejunal mucosal IFN-γ, IL-1β, and TNF-α concentrations at 42 d (Table 8, $P < 0.05$).

Table 7. Effects of dietary palygorskite-based antibacterial agent supplementation on the concentrations of serum cytokines in broiler chickens (pg/mL).

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1,2]	CON	ANT	250	500	1,000	2,000	SEM[3]	ANOVA	Linear	Quadratic
21 d										
IFN-γ	462.95	452.11	456.49	455.24	450.80	446.13	7.49	0.994	0.535	0.945
IL-1β	128.26[a]	104.56[b]	121.79[ab]	118.39[ab]	105.91[b]	101.80[b]	2.87	0.027	0.003	0.504
TNF-α	44.06[a]	35.01[b]	40.22[ab]	37.36[ab]	35.62[b]	33.69[b]	1.04	0.031	0.002	0.970
42 d										
IFN-γ	460.53[a]	417.93[bcd]	439.71[ab]	432.69[abc]	401.36[d]	394.02[d]	5.50	0.001	<0.001	0.486
IL-1β	112.36	105.08	104.42	104.33	99.32	93.23	2.46	0.354	0.033	0.784
TNF-α	40.05[a]	29.87[bc]	34.35[b]	32.45[bc]	31.41[bc]	27.71[c]	0.93	0.001	<0.001	0.874

[a,b,c,d]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, control group; ANT, antibiotic group.
[2]Abbreviations: IFN-γ, interferon-γ; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α.
[3]SEM = Standard error of the mean (n = 8).

Table 8. Effects of dietary palygorskite-based antibacterial agent supplementation on the concentrations of intestinal mucosal cytokines in broiler chickens (pg/mg protein).

			Palygorskite-based antibacterial agent level (mg/kg)					*P* values		
Items[1,2]	CON	ANT	250	500	1,000	2,000	SEM[3]	ANOVA	Linear	Quadratic
21 d										
Jejunum										
IFN-γ	211.30[a]	194.64[ab]	206.27[a]	203.12[ab]	193.87[ab]	184.58[b]	2.66	0.038	0.002	0.275
IL-1β	111.33	103.73	109.15	106.05	100.47	98.89	1.86	0.346	0.029	0.627
TNF-α	36.08[a]	26.89[bc]	31.00[ab]	27.15[bc]	25.79[bc]	22.84[c]	1.14	0.011	<0.001	0.905
Ileum										
IFN-γ	257.15	245.51	251.08	241.71	238.29	236.65	5.72	0.920	0.249	0.888
IL-1β	101.73[a]	84.12[b]	92.06[ab]	91.74[ab]	81.56[b]	77.62[b]	2.45	0.049	0.001	0.718
TNF-α	41.13[a]	33.48[ab]	37.83[ab]	33.78[a]	28.95[b]	27.91[b]	1.19	0.005	<0.001	0.702
42 d										
Jejunum										
IFN-γ	212.06[a]	178.33[b]	191.46[ab]	190.13[ab]	179.99[b]	170.39[b]	3.66	0.014	0.001	0.97
IL-1β	111.84	97.29	106.31	101.43	96.45	95.03	2.45	0.315	0.033	0.976
TNF-α	32.43[a]	23.85[bc]	27.9[ab]	25.57[bc]	22.1[cd]	18.97[d]	0.88	<0.001	<0.001	0.544
Ileum										
IFN-γ	252.31	235.21	246.12	242.5	232.97	228.27	4.95	0.759	0.150	0.775
IL-1β	97.45	86.69	95.29	88.43	84.02	80.89	2.27	0.246	0.012	0.631
TNF-α	37.72[a]	28.76[bc]	34.23[ab]	29.11[bc]	25.80[c]	24.33[c]	1.03	<0.001	<0.001	0.817

[a,b,c,d]Means within a row with different superscripts are different at $P < 0.05$.
[1]CON, control group; ANT, antibiotic group.
[2]Abbreviations: IFN-γ, interferon-γ; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α.
[3]SEM = Standard error of the mean (n = 8).

Compared with the control group, PAA supplementation at a level of 2,000 mg/kg reduced jejunal mucosal IFN-γ level at 21 d of age ($P < 0.05$). Broilers fed a basal diet supplemented with antibiotic had lower levels of jejunal mucosal TNF-α and ileal mucosal IL-1β at 21 d and jejunal mucosal IFN-γ and TNF-α and ileal mucosal TNF-α at 42 d when compared with control group ($P < 0.05$). Dietary supplementation with 1,000 and 2,000 mg/kg PAA reduced ileal mucosal IL-1β and TNF-α concentrations at 21 d and jejunal mucosal IFN-γ concentration at 42 d when compared with the control group ($P < 0.05$), and their values in these PAA-supplemented groups were similar to antibiotic group ($P > 0.05$). Likewise, compared with the control group, PAA administration from 500 to 2,000 mg/kg reduced jejunal mucosal TNF-α concentrations at both 21 and 42 d and ileal mucosal TNF-α concentrations at 42 d ($P < 0.05$), and the value of these parameters in the PAA-supplemented groups were comparable with the antibiotic group ($P > 0.05$), and the 42-d jejunal mucosal TNF-α concentration in 2,000 mg/kg PAA-supplemented group was even lower than that of antibiotic group ($P > 0.05$).

DISCUSSION

The beneficial consequences of dietary clay mineral-based antibacterial agent supplementation were reported previously in both poultry and swine. In broilers, Tang et al. (2014b) found that feeding a zinc-bearing clinoptilolite could linearly increase weight gain of broiler chicks during the early grower period, and its supplementation could even totally replace chlortetracycline to promote growth performance. Moreover, Wang et al. (2012) reported that zinc-bearing clinoptilolite as an alternative to chlortetracycline can even alleviate *Salmonella pullorum*-induced compromised growth performance in broiler chicks at an early age. As for piglets, Song et al. (2013) observed that dietary copper-exchanged calcium-montmorillonite and copper-exchanged sodium-montmorillonite administration at an equivalent amount (1.5 g/kg) both improved weight gain and feed conversion efficiency of weanling piglets, and the growth performance of piglets receiving copper-exchanged montmorillonite, irrespective of its form, was comparable with their counterparts fed antibiotic. Likewise, Ke et al. (2014) showed that a cetylpyridinium-montmorillonite supplementation linearly and quadratically increased ADG of weaned piglets, and the piglets fed 1.0 g/kg and 1.5 g/kg cetylpyridinium-montmorillonite did not differ from those fed chlortetracycline in terms of growth performance. In agreement with these findings, dietary PAA incorporation quadratically improved feed utilization efficiency of birds during the finisher and entire periods in this study, and birds fed a basal diet supplemented with 1,000 mg/kg PAA had the lowest overall FCR among PAA-supplemented treatments, which also did not differ from the antibiotic group. These findings, in turn, indicated that dietary PAA administration could improve growth performance of broiler chickens and may be used as an alternative to feed antibiotics. Available studies have demonstrated that clay mineral based-antibacterial agent could improve animal growth performance through improving nutrient digestion and absorption (Xia et., 2004; Hu et al., 2012a; Tang et al., 2014a), intestinal mucosal barrier function (Hu et al., 2012b; Jiao et al., 2015; Li et al., 2021), immune response (Hu et al., 2013; Tang et al., 2014b; Jiao et al., 2018), antioxidant capacity (Tang et al., 2014b; Jiao et al., 2018), and intestinal microflora composition (Ke et al., 2014; Tang et al., 2014b; Li et al., 2021), which may contribute to the

improved feed conversion efficiency of PAA-treated broiler chickens in the current research. The key ingredients of PAA used in this study were QACOS/ZnO/Pal nanocomposite and essential oil/Pal nanocomposite. These compositions of PAA including Pal, QACOS, ZnO, and essential oil possess multiple biological activities, including antibacterial, antioxidant, anti-inflammatory, and immunomodulatory activities (Sirelkhatim et al., 2015; Zeng et al., 2015; Juárez et al., 2016; López-Pacheco et al., 2017; Horky et al., 2019; Hui et al., 2020b; Zhong et al., 2021), and these characteristics would provide further explanations for its growth promoting effect on broiler chickens. In the current study, birds fed a basal diet supplemented with 2,000 mg/kg PAA actually possessed the highest FCR during the late and overall experimental periods, suggesting that high level of PAA would impair the normal growth of broiler chickens. The increased FCR may be associated with the supplemental level of PAA. Although the highest level of Pal used as anti-caking agent that is permitted by EU in animal feed is 20 g/kg, an in vivo study conducted in broiler chickens showed that dietary Pal supplementation at a level of 20 g/kg increased FCR of broiler chickens during the finisher and entire experimental periods due to its nutrient dilution effect (Zhang et al., 2017).

Intestinal health is vital for the growth, general health, and welfare of poultry and livestock, and intestinal mucosal barrier plays a critical role in the maintenance of intestinal health (Ducatelle et al., 2018). The intestinal mucosal barrier mainly consisting of different types of epithelial cells that are connected by tight junction proteins and protected by an overlying host-secreted mucous layer is the first defense line of the host against invading pathogenic bacteria, antinutritional factors, gastric acid, and other toxic and harmful substances (Martens et al., 2018). D-lactate is produced in minimal quantities by animal cells, and the circulating D-lactate concentration is normally maintained at a very low concentration under normal physiological conditions (Ewaschuk et al., 2005; Levitt and Levitt, 2020). However, D-lactate produced by indigenous bacteria within gastrointestinal tract under specific circumstances such as enteric infections will enter into blood circulation through the damaged intestinal barrier (Ducatelle et al., 2018; Pohanka, 2020). The endotoxin is released from bacteria during infection or as a consequence of bacterial lysis, which would translocate across the intestinal lumen into the blood circulation when intestinal barrier permeability raises (de Punder and Pruimboom, 2015). In the current study, PAA linearly decreased circulating endotoxin level at 42 d and D-lactate level at both 21 and 42 d, and the 1,000 and 2,000 mg/kg PAA administration significantly reduced 42-d plasma endotoxin and D-lactate concentrations. In addition, dietary supplementation with PAA also improved the small intestinal morphology of broiler chickens. In detail, the addition of PAA to a basal diet linearly decreased crypt depth and linearly increased the ratio of villus height and crypt depth in the intestine of broilers at 42 d of age. Moreover, the 1,000 mg/kg PAA decreased jejunal crypt depth, while 500 and 1,000 mg/kg PAA increased the ratio of jejunal villus height and crypt depth at 42 d, with their values being comparable with the antibiotic group. These findings together suggested that dietary supplementation with PAA could improve intestinal mucosal barrier integrity and function of broiler chickens. Accordingly, Hu et al. (2012b) observed that dietary montmorillonite-zinc oxide hybrid administration reduced plasma D-lactate concentration and diamine oxidase activity and increased villus height and the ratio between villus height and crypt depth in the jejunal mucosa of weaned piglets. Similarly, Song et al. (2013) reported that copper-exchanged montmorillonite as a replacement for antibiotic growth promoter decreased circulating diamine oxidase activity and D-lactate concentration of weanling pigs. Moreover, Ke et al. (2014) found that a cetylpyridinium-montmorillonite linearly and quadratically increased villus height and the ratio of villus height to crypt depth in the jejunum and decreased plasma diamine oxidase activity of weanling pigs. In broilers, Wang et al. (2012) also observed that a clinoptilolite-based antibacterial agent (zinc-bearing clinoptilolite) reduced serum diamine oxidase activity of young broiler chicks challenged with *Salmonella*. These findings, together with the results of the current study, indicated that clay mineral-based antibacterial agent, regardless of their types, could protect the intestinal mucosal barrier integrity and function of animals. The improved intestinal barrier function of broilers ingesting PAA observed in this study could be partially attributed to its antibacterial activity. Recent studies have demonstrated that the 2 main compositions of PAA, QACOS/ZnO/Pal nanocomposite and essential oil/Pal nanocomposite, both exhibited favorable in vitro antibacterial activities against *Escherichia coli* and *Staphylococcus aureus* (Hui et al., 2020b; Zhong et al., 2021). Also, the regulatory effects of Pal on the bacteria metabolism and survival through adsorption may also contribute to the improved intestinal barrier. An in vitro study demonstrated that Pal could effectively reduce respiration of the pathogenic bacteria (*Histoplasma capsulatum*) by adhering to the mycelial surface and, thereby, interfered with the movement of nutrients, metabolites, and gases across the mycelial wall (Lavie and Stotzky, 1986.) In contrast, the adsorption pattern of a probiotic (*Lactobacillus acidophilus*) on Pal was a spontaneous and endothermic process, and this adsorption could efficiently improve survivals of *Lactobacillus acidophilus* cells at simulated conditions of gastric pH and at high bile salt concentrations when compared with free bacteria (Zhao et al., 2017). In laying pullets, Chalvatzi et al. (2016) proved that birds receiving Pal had a more homogeneous cecal microbial profile and a more favorable microbiota consisting of bacteria that were the major degraders of resistant polysaccharides and efficient in butyrate production. The Pal and essential oils in PAA can maintain intestinal barrier integrity by regulating the expression of intestinal tight junction

proteins and intestinal microflora as well as mucosal immunity (Zhang et al., 2013; Chen at al., 2016a,b, 2020b; Liu et al., 2018; Omonijo et al., 2019; Yang et al., 2019; Xu et al., 2020; Ibrahim et al., 2021), which may also account for the improved intestinal barrier function in this study Moreover, the PAA-induced decreases in the concentrations of inflammatory cytokines in serum and intestinal mucosa would also contribute to the improved mucosal barrier, since inflammatory cytokines such TNF-α and IFN-γ would impair intestinal barrier function and integrity directly, as summarized previously (Groschwitz and Hogan, 2009).

In this study, the administration of PAA linearly increased the relative weight of bursa of Fabricius in broiler chickens at 21 d, and this result, in turn, suggested that PAA supplementation was beneficial for the growth and development of bursa of Fabricius, which may be associated the compositions of PAA that have immunomodulatory activities including Pal (Cervini-Silva et al., 2015; Juárez et al., 2016; López-Pacheco et al., 2017) and essential oils (Zeng et al., 2015), even though the underlying mechanism was unknown. The sIgA in the defense of mucosal epithelia plays a crucial role in preventing pathogenic bacterial adhesion to the host cells, therefore blocking dissemination and inhibiting subsequent infections (Corthésy, 2010). The IgM plays a vital role in both humoral and mucosal immunity, and it is the first class of antibody produced after B cell activation (Li et al., 2020). Chen et al. (2016b) found that dietary supplementation with a purified Pal increased sIgA and IgM concentrations in the ileal mucosa of broiler chickens at an early age. In laying hens, Chen et al. (2020a) reported dietary supplementation with montmorillonite increased the concentration of sIgA in the duodenum of laying hens. In accordance with these findings, the administration of PAA linearly and/or quadratically increased sIgA, IgM, and IgG concentrations in the intestinal mucosa at 42 d, and the 2,000 mg/kg PAA increased jejunal mucosal sIgA and IgM concentrations in broilers at 42 d of age, when compared with the control and antibiotic groups. There are several possible explanations accounting for the increased concentrations of immunoglobulins in the intestinal mucosa. The increased mucosal immunoglobulin concentration would be correlated with the antibacterial activity of PAA. Moreover, the key chemical composition of Pal, aluminosilicate, possesses immune regulatory activities, which has been shown to promote antibody production and improve immune organ development and function (Jung et al., 2010). Additionally, the elevated concentrations of intestinal mucosal immunoglobulins may be also associated with the key component of PAA, essential oils. Available literature has shown that essential oils could promote the synthesis of immunoglobulins in the small intestine of animals (Placha et al., 2014; Liu et al., 2017; Su et al., 2021). The anti-inflammatory characteristics of clay mineral-based antibacterial agents have been found previously. Jiao et al. (2018) reported that copper/zinc-loaded montmorillonite administration decreased small intestinal mucosal IL-1β, IL-6, and TNF-α levels in weanling piglets. Similarly, Song et al. (2013) also observed that copper-exchanged calcium-montmorillonite and copper-exchanged sodium-montmorillonite administration reduced IL-6 and TNF-α levels in the ileal mucosa of weaned piglets. Moreover, Hu et al. (2013) found that dietary administration with the zinc oxide supported on zeolite decreased mRNA expression levels of TNF-α and INF-γ in the jejunal mucosa of weaned pigs. In our study, dietary supplementation with PAA linearly and/or quadratically reduced the levels of inflammatory cytokines (IL-1β, INF-γ, and TNF-α) in serum and jejunal and ileal mucosa of broiler chickens, and the anti-inflammatory effect of PAA was comparable with the antibiotic to a certain extent, suggesting that PAA as an alternative to antibiotic could also inhibit the inflammatory response of broilers fed an antibiotic-free diet. The ant-inflammatory effect would be in correlation with the antibacterial characteristic of PAA (Hui et al., 2020b; Zhong et al., 2021). The carrier of PAA, Pal, has also been shown to exhibit anti-inflammatory activity. In rodent animal models, studies demonstrated that Pal could inhibit inflammation (Juárez et al., 2016; López-Pacheco et al., 2017), and it was believed to be correlated with its unique fibrous structure. Cervini-Silva et al. (2015) found that the fibrous clay structure of Pal could restrict the kinetics and mechanism of myeloperoxidase inhibition, which, in turn, endowed it anti-inflammatory property. Moreover, the fibrous structure of Pal could alter the infiltration of different leukocyte cells to an inflammation site, eventually leading to presence of few macrophages at the inflammation site and the mitigation of inflammation (Juárez et al., 2016). In an in vivo study, Chen et al. (2020b) reported that Pal administration inhibited lipopolysaccharide-induced intestinal mucosal and systemic inflammatory responses of young broiler chickens through decreasing the synthesis of proinflammatory cytokines at both protein and mRNA expression levels. Meanwhile, it is also necessary to mention that the anti-inflammatory effect of essential oils, the effective components of PAA, would also provide an explanation for the decreases in the concentrations of proinflammatory cytokines observed in this study, as summarized previously (de Cássia da Silveira e Sá et al., 2014; Valdivieso-Ugarte et al., 2019; Gandhi et al., 2020). A theory on the allocation of available energy resources and nutrients to either growth or immune system has been proposed (Qureshi and Havenstein, 1994; Spurlock, 1997). Thus, the improved growth performance of PAA-treated broiler chickens may be closely associated with its anti-inflammatory effects, similar to the growth-promoting effects of antibiotics.

In conclusion, this study suggested that dietary supplementation with PAA could improve feed conversion efficiency, intestinal mucosal barrier function and integrity, and improve immunity of broiler chickens, and may be used as a potential alternative to antibiotics, with its optimal level being 1,000 mg/kg in broiler feed in the current research.

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DISCLOSURES

The author(s) declare(s) no conflicts of interest.

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