JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0180041

Palygorskite Supplementation Improves Growth Performance, Oxidative Status, and Intestinal Barrier Function in Cherry Valley Ducks

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The effects of dietary palygorskite (Pal) supplementation on growth performance, oxidative status, and intestinal barrier function in ducks were investigated. In total, 720 one-day-old Cherry Valley ducks were categorized into 4 treatments comprising 6 replicates with 30 ducks each. Ducks were fed a basal diet supplemented with 0, 5, 10, or 20 g/kg Pal for 42 days. Twenty-four ducks (1 male/replicate) were slaughtered at 14 and 42 days and samples were collected for analysis. Pal supplementation quadratically increased weight gain and linearly and quadratically increased feed intake ($P<0.05$) during the starter period. Pal enhanced serum glutathione peroxidase activity (GSH-Px) at 14 (linear and quadratic, $P<0.05$) and 42 days (linear, $P<0.001$), and lowered serum malondialdehyde (MDA) content at 14 and 42 days (quadratic, $P<0.05$). It enhanced 42-day liver superoxide dismutase activity (linear, $P=0.003$) and GSH-Px activity at 14 (quadratic, $P=0.044$) and 42 days (linear and quadratic, $P<0.001$), but decreased 14-day liver MDA content (quadratic, $P=0.003$). Pal reduced 42-day serum diamine oxidase activity (linear and quadratic, $P<0.05$) and serum endotoxin content at 14 (linear and quadratic, $P<0.05$) and 42 days (quadratic, $P=0.017$). It linearly and quadratically increased jejunal mucosal immunoglobulin (Ig) M at 42 days and IgG at 14 and 42 days, and 42-day ileal mucosal IgG and secretory IgA ($P<0.05$). Ileal mucosal IgM content was quadratically increased at 14 and 42 days ($P<0.05$) by Pal. Moreover, Pal enhanced the mRNA expression of 14-day occludin in the jejunal mucosa (quadratic, $P=0.033$) and that of 42-day zonula occludens-1 in the ileal mucosa (linear, $P=0.027$). Thus, dietary Pal supplementation exerts beneficial effects through improving growth performance, antioxidant capacity, and intestinal barrier function of ducks.

Key words: barrier function, Cherry Valley ducks, growth performance, oxidative status, palygorskite

J. Poult. Sci., 56: 186-194, 2019

Introduction

Palygorskite (Pal) is a natural magnesium aluminum silicate clay mineral comprised of ribbons of a 2:1 phyllosilicate unit ($Si_8Mg_8O_{20}(OH)_2 \cdot 4H_2O$) (Galán, 1996; Murray, 2000). Owing to its unique physicochemical properties, including small partical size and a fibrous structure with micropores and channels (Galán, 1996; Zhou, 2011; Xu *et al*., 2012), Pal has a high absorption capacity, colloidal and swelling capacity, optimal rheological behavior, and high water dispersibility (Ghadiri *et al*., 2015). Pal is applicable in various industries as well as in animal nutrition (Murray, 2000; Chalvatzi *et al*., 2014).

Recently, Pal has been gradually adopted in animal production either as a feed pellet binder or as animal feed supplement because of its aforementioned characteristics (Murray, 2000; Yan *et al*., 2016). Dietary Pal supplementation has been reported to enhance feed pellet quality and to increase the average daily gain in broilers (Zhang *et al*., 2017). It has been suggested that Pal addition improves immunity oxidative status, intestinal integrity, and digestive function of broilers (Chen *et al*., 2016a, b). Supplementation of dietary Pal in weaned piglets reportedly improves growth performance while reduces the diarrhea rate, thus improving intestinal integrity (Zhang *et al*., 2013; Lv *et al*., 2015). In addition, Pal supplementation improves laying hen performance and egg quality (Chalvatzi *et al*., 2014), and the milk yield of lactating Holstein cows and milk quality of cows and ewes (Bampidis *et al*., 2014; Kotsampais *et al*., 2017).

Considering the antioxidant effects and protective roles of dietary Pal supplementation in those animal models, we reasoned that Pal supplementation might benefit ducks as well. Therefore, this study aimed to determine the effects of Pal supplementation on growth performance, antioxidant capacity, and intestinal barrier function of Cherry Valley

Received: March 28, 2018, Accepted: September 3, 2018
Released Online Advance Publication: November 25, 2018
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ducks.

Materials and Methods

Pal

Pal was obtained from Jiangsu Sinitic Biotech Co., Ltd. (Xuyi, Jiangsu, China). The main chemical constituents of Pal as determined with a Minipal 4 X-ray fluorescence spectrometer (PANanalytical Co., Almelo, Netherlands) were: SiO_2, 56.32%; Al_2O_3, 10.25%; MgO, 13.32%; CaO, 10.14 %; K_2O, 1.15%; Na_2O, 0.77% and Fe_2O_3, 6.02%.

Animals, Diets, and Experimental Design

All procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee. For the trial, 720 one-day-old Cherry Valley ducks (obtained from a commercial hatchery) having a similar initial body weight (50.00±0.20 g) were randomly allocated to 4 dietary treatments. Each treatment consisted of 6 replicates with 30 ducks (15 males and 15 females per replicate). The ducks were fed with a basal diet supplemented with 0 (control group), 5, 10, or 20 g/kg Pal for 42 days. The basal diet was formulated according to Nutrient Requirements of Meat-Type Duck of China (NY/T 2122-2012). The composition and nutrient content of the basal diet are listed in Table 1. Ducks were allowed *ad libitum* access to pellet feed and water on a plastic slatted floor in a temperature-controlled room with continuous lighting. The temperature was maintained at 32−33℃ for the first 3 days and was then gradually decreased by 2−3℃ per week to a final temperature of 26℃. The experimental period consisted of a starter period (1 to 14 days) and a grower period (15 to 42 days). At 14 and 42 days, ducks were weighed in replicate after feed deprivation for 12 h, and feed intake was recorded in replicate to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G).

Sample Collection

At 14 and 42 days, 24 randomly selected male ducks (1 duck per replicate) were weighed after feed deprivation for 12 h. Blood samples (5 mL) taken from the wing vein were centrifuged at 4 450×*g* for 15 min at 4℃ to obtain serum, which was stored at −28℃ until analysis. After blood collection, the ducks were immediately euthanized by cervical dislocation and necropsied. The liver samples were quickly excised, frozen in liquid nitrogen, and stored at −80℃ for antioxidant capacity assay. The bursa of Fabricius, thymus,

Table 1. **Composition and nutrient level of basal diet (g/kg, as fed basis)**

Item	1−14 days	15−42 days
Ingredient		
Corn	500	520
Wheat	30	100
Wheat midding	110	80
Cottonseed meal	50	60
Soybean meal	80	50
Corn gluten meal	100	70
Meat and born meal	30	30
Rice bran	65	51
L-Lysine	1.5	2
DL-Methionine	1.5	2
Limestone	14	12
Dicalcium phosphate	5	10
Sodium chloride	3	3
Premix[a]	10	10
Calculated nutrient level		
Apparent metabolizable energy (MJ/kg)	12.13	12.34
Crude protein	193.8	171.9
Calcium	9.5	10.0
Available phosphorus	4.0	3.9
Lysine	11.0	7.8
Methionine	4.5	4.3
Methionine + cystein	7.7	7.1
Analyzed nutrient composition		
Crude protein	195.9	175.0
Crude ash	62.1	77.0
Calcium	9.4	9.7
Total phosphorus	11.3	11.7

[a] Premix provided per kg diet: vitamin A 10000 IU; vitamin D 2000 IU; vitamin E 20 IU; vitamin K 0.5 mg; vitamin B_{12} 0.04 mg; nicotinic acid 60 mg; D-pantothenie 11 mg; pyridoxine 2.5 mg; riboflavin 4.0 mg; biotin 0.2 mg; folic acid 0.6 mg; thiamine 3 mg; choline chloride 600 mg; Cu 8 mg; Fe 80 mg; Mn 80 mg; Zn 60 mg; Se 0.2 mg; I 0.4 mg.

and spleen were collected and weighed to calculate the relative organ weights using the following formula: relative immune organ weight (g/kg)＝immune organ weight (g) / body weight (kg). The whole gastrointestinal tract was rapidly removed. The jejunum and ileum were collected and were flushed with ice-cold phosphate-buffered saline. Approximately 20 cm of jejunal and ileal segments were opened along the longitudinal axis. The mucosa were scratched carefully using a sterile glass microscope slide, quickly frozen in liquid nitrogen, and stored at −80℃ until analysis.

Measurements of Serum and Liver Antioxidant Indexes

Antioxidant indexes of serum and liver, including superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px) activities and the malondialdehyde (MDA) level, were determined using commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) according to the manufacturer's instructions.

The serum was diluted to the appropriate concentration after defrosting. Then, the SOD and GSH-Px activities and MDA level were measured and were expressed as U/mL and nmol/mL serum, respectively.

Liver tissue (0.3 g) was mixed (1:4, W/V) with cold sodium chloride solution (154 mmol/L, 4℃) and was homogenized by using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH, USA). The suspensions obtained were centrifuged at 4 450×*g* for 15 min at 4℃. The supernatant was collected and evaluated for SOD and GSH-Px activities and the MDA level. Protein concentration was determined by the Bradford method (1976). Data were normalized against the total protein concentration in each sample for inter-sample comparison.

Determination of Diamine Oxidase Activity and Endotoxin Content in the Serum

The activity of diamine oxidase in the serum was determined according to the method described by Chen *et al*. (2016a). The content of endotoxin in the serum was determined using Chromogenic End-point Tachchypleus Amebocyte Lysate obtained from Xiamen Limulus Reagent Biotechnology Co., Ltd. (Xiamen, China).

Mucosal Immune Parameters

Samples of ileal and jejunal mucosa (0.3 g) were homogenized (1:4, W/V) with cold sodium chloride solution (154 mmol/L, 4℃) using an Ultra-Turrax homogenizer (Tekmar Co.). The homogenates were centrifuged at 4 450×*g* for 15 min at 4℃, and the supernatants were collected and stored at −28℃ for subsequent determination. The levels of immunoglobulin M (IgM), immunoglobulin G (IgG), and secretory immunoglobulin A (SIgA) were measured using duck-specific IgG, IgM, and SIgA enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). The concentrations of IgM, IgG, and SIgA in the intestinal mucosa were standardized to the concentration of protein in each sample.

Quantitative Reverse-transcription PCR

Briefly, mucosal RNA was extracted using Trizol reagent (Takara Biotechnology, Dalian, Liaoning, China), following the manufacturer's instructions. RNA integrity was verified by gel electrophoresis followed by ethidium bromide staining. Total RNA was quantified by measuring the absorbance at 260 nm, and RNA purity was assessed by determining the ratio of absorbance at 260 and 280 nm using a NanoDrop ND-2000 spectrophotometer (Nano Drop Technologies, Wilmington, DE, USA). The RNA was reverse-transcribed to cDNA using a PrimeScript^{TM} RT reagent Kit (Takara Biotechnology, Dalian, Liaoning, China). Real-time PCR was carried out with the ABI StepOnePlus^{TM} Real-Time PCR system (Applied Biosystems, Grand Island, NY, USA) using the following thermal cycling protocol: 95℃ for 30 s followed by 40 cycles of 95℃ for 5 s, 60℃ for 31 s, and a final dissociation stage of 95℃ for 15 s, 60℃ for 1 min, 95℃ for 15 s, and 60℃ for 15 s. *β-Actin* was adopted as a reference gene. The primer sequences for the target [zonula occludens-1 (*ZO-1*), occludin (*OCLN*), claudin-1 (*CLDN-1*)] and reference genes are listed in Table 2. The $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen, 2001) was used to analyze the relative expression (fold changes), calculated relative to the control group. The result of the control group was defined as 1.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (Ver. 20.0 for windows; SPSS Inc., Chicago, IL, USA). The linear and quadratic effects of dietary Pal supplementation levels were determined by polynomial contrasts. Data are presented as means and their total standard errors. Significance was considered at $P<0.05$.

Table 2. **Information on primers used for real-time PCR**

Gene name[a]	Gene Bank ID	Primer sequence (5′ → 3′)	Length
ZO-1	XM_413773.4	Forward: TGTAGCCACAGCAAGAGGTG Reverse: CTGGAATGGCTCCTTGTGGT	159
OCLN	NW_004679913.1	Forward: CTCTGCTTCCTGGCCCAGTT Reverse: AGACGATGGAGGCGATGAGC	214
CLDN-1	NW_004678814.1	Forward: TCCATGCATGTGCTGTTGGC Reverse: CCTGCTGCAGTTGCAGTGTT	145
β-Actin	NM_205518.1	Forward: TTGGTTTGTCAAGCAAGCGG Reverse: CCCCCACATACTGGCACTTT	100

[a] *ZO-1*, zonula occludens-1; *OCLN*, occludin; *CLDN-1*, claudin-1.

Results

Growth Performance

Pal supplementation (Table 3) quadratically increased ADG ($P=0.003$) and linearly and quadratically promoted ADFI ($P<0.05$) of ducks during the starter period, whereas it linearly decreased ADG ($P=0.042$) of ducks during the grower period.

Relative Immune Organ Weight

As indicated in Table 4, dietary Pal supplementation had no effect on the relative weights of the thymus, spleen, and bursa of Fabricius ($P>0.05$) at 14 and 42 days.

Serum and Liver Oxidative Status

Serum GSH-Px activity was enhanced by Pal supplementation at 14 days (linear and quadratic, $P<0.05$) and 42 days (linear, $P<0.001$). In contrast, the MDA content was quadratically reduced by Pal supplementation at 14 and 42 days ($P<0.05$) (Table 5). As for the liver, Pal supplementation linearly ($P=0.003$) increased SOD activity at 42 days. Meanwhile, GSH-Px activity was enhanced by Pal supplementation at 14 days (quadratic, $P=0.044$) and 42 days (linear and quadratic, $P<0.001$). However, the MDA content was quadratically ($P=0.003$) decreased by Pal supplementation at 14 days.

Diamine Oxidase Activity and Endotoxin Content in the Serum

As indicated in Table 6, serum diamine oxidase activity was linearly and quadratically decreased ($P<0.05$) by Pal supplementation at 42 days. Similarly, the content of endotoxin was reduced by Pal supplementation at 14 days (linear and quadratic, $P<0.05$) and 42 days (quadratic, $P=0.017$), respectively.

Intestinal Immunoglobulin

Supplementation of Pal linearly and quadratically enhanced IgM content at 42 days ($P<0.05$) and IgG content at 14 and 42 days in the jejunal mucosa ($P<0.05$) (Table 7). Meanwhile, Pal supplementation quadratically increased the IgM content of the ileal mucosa at 14 and 42 days ($P<0.05$). IgG and SIgA contents of ileal mucosa were linearly and quadratically ($P<0.05$) elevated at 42 days after Pal incorporation.

Table 3. **Effect of palygorskite supplementation on the growth performance of ducks from 1 to 42 days**

Item[a]	Palygorskite (g/kg)				SEM[b]	*P*	
	0	5	10	20		Linear	Quadratic
ADG (g/day/duck)							
1–14 days	32.67	35.85	40.10	34.72	0.82	0.081	0.003
15–42 days	84.88	83.68	82.76	82.37	0.45	0.042	0.651
1–42 days	67.48	67.74	68.85	66.76	0.37	0.750	0.120
ADFI (g/day/duck)							
1–14 days	39.16	43.91	47.04	42.76	0.71	0.002	<0.001
15–42 days	179.40	176.10	180.18	176.19	1.92	0.754	0.939
1–42 days	132.69	132.04	135.80	131.72	1.35	0.948	0.547
F:G							
1–14 days	1.20	1.23	1.18	1.24	0.02	0.724	0.789
15–42 days	2.12	2.11	2.18	2.14	0.02	0.481	0.789
1–42 days	1.96	1.95	1.97	1.98	0.02	0.712	0.721

[a] ADG, average daily gain; ADFI, average daily feed intake; F:G, feed/gain ratio.
[b] SEM, total standard error of means.

Table 4. **Effect of palygorskite supplementation on relative immune organ weight of male ducks**

Item	Palygorskite (g/kg)				SEM[a]	*P*	
	0	5	10	20		Linear	Quadratic
Thymus (g/kg body weight)							
14 days	4.35	4.10	3.83	4.23	0.10	0.464	0.116
42 days	3.35	3.41	2.88	3.24	0.09	0.284	0.400
Spleen (g/kg body weight)							
14 days	0.99	0.87	0.87	0.94	0.04	0.885	0.085
42 days	0.66	0.63	0.60	0.60	0.03	0.445	0.816
Bursa of Fabricius (g/kg body weight)							
14 days	1.23	1.26	1.29	1.31	0.02	0.117	0.886
42 days	0.60	0.78	0.79	0.69	0.04	0.480	0.130

[a] SEM, total standard error of means.

Table 5. **Effect of palygorskite supplementation on the antioxidant status in the serum and liver of male ducks**

Item[a]	Palygorskite (g/kg)				SEM[b]	*P*	
	0	5	10	20		Linear	Quadratic
Serum							
SOD (U/mL)							
14 days	30.58	33.06	31.52	30.77	0.68	0.880	0.263
42 days	26.94	27.23	27.19	27.70	0.60	0.697	0.932
GSH-Px (U/mL)							
14 days	661.26	813.66	807.16	806.28	20.84	0.011	0.038
42 days	716.05	828.27	838.89	925.21	21.75	<0.001	0.697
MDA (nmol/mL)							
14 days	3.88	2.19	3.60	3.62	0.15	0.463	<0.001
42 days	3.85	2.66	3.59	3.47	0.13	0.808	0.012
Liver							
SOD (U/mg protein)							
14 days	222.89	249.21	228.65	238.51	3.53	0.443	0.215
42 days	255.00	294.50	338.93	320.73	9.84	0.003	0.081
GSH-Px (U/mg protein)							
14 days	40.16	41.65	42.20	39.03	0.57	0.564	0.044
42 days	30.71	35.55	41.17	36.40	0.84	<0.001	<0.001
MDA (nmol/mg protein)							
14 days	1.15	1.02	0.98	1.09	0.02	0.219	0.003
42 days	1.14	1.06	1.06	1.14	0.03	0.978	0.188

[a] SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; MDA, malonaldehyde.
[b] SEM, total standard error of means.

Table 6. **Effect of palygorskite supplementation on diamine oxidase activity and endotoxin content in the serum**

Item	Palygorskite (g/kg)				SEM[a]	*P*	
	0	5	10	20		Linear	Quadratic
Diamine oxidase (U/L)							
14 days	13.43	10.02	10.04	10.56	0.57	0.084	0.077
42 days	13.71	11.38	10.28	11.59	0.44	0.045	0.029
Endotoxin (EU/mL)							
14 days	0.20	0.17	0.16	0.18	0.01	0.047	0.001
42 days	0.61	0.47	0.45	0.59	0.02	0.741	0.017

[a] SEM, total standard error of means.

Intestinal Gene Expression

As shown in Table 8, Pal supplementation quadratically ($P=0.033$) elevated the mRNA level of *OCLN* in the jejunal mucosa at 14 days. mRNA expression of *ZO-1* in ileal mucosa was linearly ($P=0.027$) increased at 42 days by Pal supplementation.

Discussion

The results in the present study showed that dietary Pal supplementation increased ADG and ADFI of ducks during the starter period. These results are in agreement with the findings of Zhang *et al*. (2017), who discovered that Pal supplementation (5, 10, 15, or 20 g/kg) improved ADG and ADFI (linear and quadratic) of broilers during the starter period. Likewise, it has been reported that dietary 1% Pal supplementation improved the laying percentage and feed/eggs produced ratio of laying hens (Chalvatzi *et al*., 2014). In weaned piglets, the incorporation of 2 g/kg Pal in the diet improved growth performance by reducing the feed/gain ratio, whereas 3 g/kg Pal showed no effect (Zhang *et al*., 2013). In contrast, neither 2.0% natural Pal nor heat-modified Pal supplementation caused desirable effects on laying performance (Qiao *et al*., 2015), and 0.5 mass% or 1.0 mass % Pal inclusion did not affect body mass, feed intake, and feed/gain ratio in broilers (Chen *et al*., 2016b). The growth performance of broilers was not significantly improved by dietary Pal supplementation in a study by Cheng *et al*. (2016b). These inconsistent results suggest that the effect of

Table 7. **Effect of palygorskite supplementation on immunoglobulin concentrations in intestinal mucosa of male ducks**

Item[a]	Palygorskite (g/kg)				SEM[b]	*P*	
	0	5	10	20		Linear	Quadratic
Jejunum							
IgM (μg/mg protein)							
14 days	7.71	7.61	8.12	7.90	0.08	0.126	0.692
42 days	7.19	7.46	8.77	7.67	0.18	0.031	0.019
IgG (μg/mg protein)							
14 days	9.74	10.37	13.00	11.22	0.33	0.002	0.016
42 days	10.51	10.54	15.64	11.51	0.50	0.003	0.001
SIgA (μg/mg protein)							
14 days	0.66	0.68	0.66	0.68	0.01	0.578	0.980
42 days	0.40	0.40	0.43	0.44	0.01	0.221	0.976
Ileum							
IgM (μg/mg protein)							
14 days	7.18	8.65	8.54	7.66	0.17	0.259	$<$0.001
42 days	9.06	11.23	9.85	9.36	0.29	0.816	0.016
IgG (μg/mg protein)							
14 days	12.56	13.18	13.80	12.82	0.23	0.487	0.090
42 days	14.20	18.39	20.38	16.83	0.60	0.013	$<$0.001
SIgA (μg/mg protein)							
14 days	0.85	0.91	0.93	0.90	0.02	0.224	0.167
42 days	0.89	1.00	1.07	1.00	0.02	0.018	0.021

[a] IgM, immunoglobulin M; IgG, immunoglobulin G; SIgA, secretory immunoglobulin A.
[b] SEM, total standard error of means.

Table 8. **Effect of palygorskite supplementation on the expression of barrier function-related genes in intestinal mucosa of male ducks**

Item[a]	Palygorskite (g/kg)				SEM[b]	*P*	
	0	5	10	20		Linear	Quadratic
Jejunum							
ZO-1							
14 days	1.00	1.05	1.22	1.07	0.03	0.124	0.065
42 days	1.00	1.02	0.98	1.13	0.03	0.240	0.292
OCLN							
14 days	1.00	1.24	1.10	1.04	0.04	0.943	0.033
42 days	1.00	1.07	1.04	1.09	0.05	0.673	0.917
CLDN-1							
14 days	1.00	1.04	1.08	1.02	0.02	0.672	0.324
42 days	1.00	0.96	1.05	1.05	0.02	0.158	0.545
Ileum							
ZO-1							
14 days	1.00	1.12	1.28	1.17	0.04	0.075	0.162
42 days	1.00	1.03	1.19	1.13	0.03	0.027	0.347
OCLN							
14 days	1.00	0.98	1.02	1.06	0.05	0.636	0.781
42 days	1.00	1.01	1.07	1.02	0.04	0.752	0.771
CLDN-1							
14 days	1.00	1.05	1.06	0.99	0.05	0.980	0.595
42 days	1.00	1.13	1.24	1.16	0.05	0.167	0.268

[a] *ZO-1*, zonula occludens-1; *OCLN*, occludin; *CLDN-1*, claudin-1.
[b] SEM, total standard error of means.

Pal supplementation on animal growth performance may depend on animal species, duration of supplementation, and the dosage used. There are several explanations why Pal improved animal performance: Pal might absorb adverse factors in the feed, thus reducing intestinal impairment and preventing gastrointestinal upsets (Slamova *et al*., 2011). By taking a Pal-containing diet, the intestinal morphology can be improved (Qiao *et al*., 2015; Chen *et al*., 2016a), and intestinal digestive enzyme activities can be enhanced (Qiao *et al*., 2015; Chen *et al*., 2016b). In addition, nutrient digestibility-promoting (Tang *et al*., 2000; Lv *et al*., 2015; Chen *et al*., 2016b) and antibacterial effects (Slamova *et al*., 2011) are other key functions of Pal in animal production. These factors may together contribute to the improved performance in animal production. Moreover, we observed that Pal supplementation decreased the ADG of ducks during the grower period, which may be related to ADFI and environmental factors in this period.

The thymus and bursa of Fabricius are two organs that play vital roles in cellular and humoral immunity (Sharma, 1999). The spleen is a fascinating organ that accommodates the capture and destruction of pathogens and the induction of adaptive immune responses (Mebius and Kraal, 2005). The present study revealed that dietary Pal supplementation did not affect the relative immune-organ weights in ducks. However, dietary natural clinoptilolite (an aluminum silicate clay) and modified clinoptilolite both increased the relative weights of immune organs in broiler chickens that were repeatedly challenged with lipopolysaccharide (Wu *et al*., 2013). These inconsistent results may be related to the health status of the animals. In addition, animal species and the type, usage, and amount of clay used may account for these discrepancies.

SOD and GSH-Px are two main antioxidant enzymes in the body that can remove oxygen free radicals (Wills, 1966; Ermak and Davies, 2002). As the major end product of lipid peroxidation, MDA is an indicator compound, and MDA content can be used to assess the extent of lipid peroxidation (Sumida *et al*., 1989). In the present study, we found that dietary supplementation of Pal significantly enhanced or tended to enhance GSH-Px and SOD activities and reduced MDA accumulation in the serum and liver, indicating that Pal inclusion improves the antioxidant capacity of ducks. A similar result was reported by Chen *et al*. (2016a), who reported that 10 g/kg Pal supplementation increased the intestinal T-SOD activity of broilers. In addition, the improved antioxidant capacity upon Pal supplementation in this study was in agreement with the results of Wu *et al*. (2013), who found that clinoptilolite supplementation increased the activities of SOD, GSH-Px, and catalase, whereas it decreased the MDA concentration in the livers of broilers. A recent study showed that Pal supplementation reduced oxidative stress in sows by lowering the levels of thiobarbituric acid reactive substances and ferric reducing ability of plasma (Papadopoulos *et al*., 2016). Pavelić *et al*. (2003) claimed that clay minerals such as zeolite (also known as silicate clay) can serve as reactive oxygen species scavengers. Similarly, a recent study conducted by Cervini-Silva *et al*. (2015) revealed that Pal dose-dependently inhibited thiobarbituric acid reactive substance generation by directly scavenging hydroxyl radicals, which was correlated with its surface sites, a signature of specific adsorption. Thus, this beneficial function of Pal would contribute to the improved oxidative status of birds receiving Pal administration.

In the present study, Pal supplementation increased IgM and IgG concentrations of the jejunal mucosa and IgM, IgG, and SIgA concentrations of the ileal mucosa during the experimental period. The results are in agreement with those reported by Chen *et al*. (2016a), who found that Pal supplementation elevated the concentrations of IgM and SIgA in the ileum in broilers. Islam *et al*. (2014) reported that 0.5% dietary artificial zeolite significantly increased the IgG concentration in both growing and finishing stages in pigs. Similar elevations of the serum IgG concentration have been observed in growing pigs supplemented with 0.5% and 1.0% sericite (silicate clay) (Li and Kim, 2013). Silicate, as a superantigen, can active polyclonal human T cells to improve immunity (Ueki *et al*., 1994). Likewise, Jung *et al*. (2010) found that aluminosilicate administration could increase the phagocytic activities of polymorphonuclear leucocytes, serum antibody production, and spleen B cell ratio in mice. The major chemical composition of Pal is aluminosilicate. Together, these studies suggest that the beneficial effects of Pal supplementation on immunoglobulin production may be associated with its major chemical component, aluminosilicate.

DAO is a highly active enzyme that is secreted by intestinal epithelial cells. Endotoxin is a metabolite of gram-negative bacteria and is a unique structure of their cell wall. DAO activity and endotoxin content in the blood can be used as indicators of intestinal barrier function and intestinal permeability (Luk *et al*., 1983; Ammori *et al*., 2003). Tight junction proteins, including *ZO-1*, *OCLN*, and claudins, are responsible for intestinal barrier function (Gu *et al*., 2011). Previous studies have indicated that reduced expression of *ZO-1* was correlated with increased intestinal permeability (Gu *et al*., 2011; Song *et al*., 2014). In the present study, Pal supplementation significantly decreased DAO activity and the endotoxin content in the serum, but enhanced the mRNA expression of *OCLN* in the jejunal mucosa at 14 days and that of *ZO-1* in the ileal mucosa at 42 days. These results indicated that Pal supplementation had a beneficial effect on intestinal barrier function. This was in agreement with the results of Chen *et al*. (2016a), who found that dietary Pal supplementation improved intestinal barrier function in broilers by lowering serum DAO activity and elevating the mRNA expression of intestinal *ZO-1*. Zhang *et al*. (2013) demonstrated that Pal supplementation improved intestinal barrier function in weaned piglets via decreasing endotoxin content and DAO activity in the plasma. It has been reported that minerals in clay may act as an antisecretory agent that prevents intestinal damage (Guarino *et al*., 2009). Because of its large specific surface area, Pal may cover the intestinal mucosa and thus form a barrier immediately after ingestion

(Zhang *et al.*, 2013). Moreover, More *et al*. (1992) found that Pal exerts cytoprotective effects on the intestine by modifying polysaccharide components of gastrointestinal glycoproteins, which play a key role in maintaining intestinal integrity. In addition, the capacity to bind toxins (Schell *et al*., 1993) and improve immune function (González *et al.*, 2004), and the anti-inflammatory function of Pal (Juárez *et al*., 2016; López-Pacheco *et al.*, 2017) may contribute to the improvement in intestinal permeability and intestinal barrier function as well.

Conclusions

The present study demonstrated that dietary Pal supplementation (5, 10, or 20 g/kg) increased ADG and ADFI in ducks during the starter period. Pal supplementation promoted the oxidative status of the ducks as evidenced by enhanced SOD and GSH-Px activities and reduced MDA contents in the serum and liver. DAO activity and endotoxin content in the serum were decreased, whereas the mRNA expression of *ZO-1* and *OCLN* in intestinal mucosa was increased by Pal supplementation. Thus, dietary Pal supplementation can improve growth performance, antioxidant capacity, and intestinal barrier function of ducks.

Acknowledgments

The study was supported by the open funding of the Xuyi County Government (2015−01).

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