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Effects of Palygorskite Inclusion on the Growth Performance, Meat Quality, Antioxidant Ability, and Mineral Element Content of Broilers

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Abstract The present study was conducted to investigate different levels of palygorskite supplementation on the growth performance, meat quality, muscular oxidative status, and mineral element accumulation of broilers. One hundred ninety-two 1-day-old Arbor Acres broiler chicks were allocated to four dietary treatments with six replicates of eight chicks per replicate. Birds in the four treatments were given a basal diet supplemented with 0, 5, 10 and 20 g/kg palygorskite for 42 days, respectively. Compared with the control group, neither 5 g/kg nor 10 g/kg palygorskite inclusion affected growth performance of broilers during the 42-day study ($P > 0.05$). However, the highest level of palygorskite supplementation at 20 g/kg increased feed/gain ratio (F/G) of broilers ($P < 0.001$). Yellowness ($P < 0.001$) and redness ($P = 0.003$) of breast muscle and yellowness of leg muscle ($P = 0.001$) were decreased by palygorskite supplementation at the levels of 10 g/kg and especially 20 g/kg. In addition, redness of leg muscle was also reduced by the inclusion of 20 g/kg palygorskite ($P = 0.009$). In contrast, malonaldehyde (MDA) accumulation in the breast muscle was significantly increased by 20 g/kg palygorskite supplementation ($P < 0.001$). Supplementation of palygorskite at either 10 or 20 g/kg significantly decreased lead (Pb) accumulation in the breast ($P = 0.001$) or thigh ($P = 0.045$) and copper (Cu) accumulation in the breast ($P = 0.022$). In conclusion, growth performance, meat color, and antioxidant capacity of meat would reduce with the increasing level of palygorskite supplementation, whereas a higher level of palygorskite (10 or 20 g/kg) can alter mineral element accumulations in muscles as evidenced by reduced muscular Cu and Pb contents.

Keywords Palygorskite · Growth performance · Meat color · Malondialdehyde · Mineral element · Broilers

Introduction

Palygorskite categorized as a raw feed material is widely used either as pellet binder of animal feed or as animal feed supplement in China, and its supplementation can bring beneficial consequences into animal nutrition, which is mainly due to its high adsorption capacity, large specific surface area, and rheological and catalytic properties [1]. It has been reported that palygorskite incorporation alleviated the adverse damage induced by aflatoxin-contaminated diets in weanling pigs [2]. Also, Zhang et al. [3] found that palygorskite enhanced growth performance, decreased diarrhea rate, and improved intestinal morphology and barrier function of weaned piglets. In broilers, dietary 1.0 % palygorskite incorporation, used as a pellet binder, can increase hardness of pellets without adverse effect on the growth performance of broilers during a 6-week study [4].

Pigmentation is an important factor in consumer acceptance and perceived quality of animal products as meat and eggs [5–8]. The color of egg yolks mainly originates from yellow (e.g., lutein, zeaxanthin, apo-ester) and red (e.g., canthaxanthin, citraxanthin, astaxanthin) carotenoids or xanthophylls in the diets [8]. However, clay supplementation including palygorskite to the laying hen diet can induce the reduced yolk color [9–11], and it is most likely due to their affinity for pigments [12, 13], suggesting that clay

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supplementation would decrease the accumulation of pigments in yolk. Most of the variation (86–90 %) in lightness, redness, and yellowness, chroma (saturation) and hue angle of pork of normal meat quality could be explained by the pigment content, myoglobin forms, and internal reflectance [6]. These results aforementioned together indicated that palygorskite can also alter meat color by changing muscular pigment retention due to its affinity for pigments occurred in the feed, which can in turn change oxidative status of meat since pigments as carotenoids possess antioxidant capacity [14–16].

Heavy metals as lead (Pb) and cadmium (Cd) are toxic pollutants for animals and humans, and their accumulation in the environment can raise agricultural and public concerns [17]. Accumulations of heavy metals in humans through the residues in the food chain can deleteriously impair the hematopoietic, nervous, and reproductive systems and the urinary tract [18, 19]. In vitro studies have demonstrated palygorskite used as an adsorbent exhibited a prominent capacity for the removal of Pb or Cd in aqueous solution, and heavy metal contained water or soil [20–22]. Recently, Zhang et al. [23] have shown that dietary 2 % palygorskite supplementation significantly decreased Cd accumulation in the muscle of blunt snout bream (*Megalobrama amblycephala*).

However, little was known about palygorskite inclusion on the meat quality, muscular oxidative status, and mineral element accumulation of broilers. We hypothesized that the inclusion of palygorskite to broilers diets would alter mineral element contents especially the heavy metals in muscles, whereas high level of palygorskite supplementation may impair the meat quality and muscular antioxidant capacity due to its adsorption capacity. The maximum content of clays including bentonite-montmorillonite and sepiolite used as binder and anti-caking in complete feedstuff is 20 g/kg for all animal species [24]. Therefore, we selected 20 g/kg as the highest dosage and investigated different levels of palygorskite supplementation on the growth performance, meat quality, muscular antioxidant capacity, and mineral element content of broilers.

Materials and Methods

Bird Husbandry, Diets, and Experimental Design

All procedures were approved by Nanjing Agricultural University Institutional Animal Care and Use Committee.

Palygorskite was kindly gifted by respected Dr. Wang in the center of Eco-materials and Green Chemistry, Lanzhou Institute of Chemical Physics, Chinese Academy of Sciences, Lanzhou, P. R. China. The main chemical compositions of palygorskite measured by a Minipal 4X-ray fluorescence spectrometer (PANalytical, Netherlands) were shown in the following: SiO_2, 52.29 %; Al_2O_3, 12.29 %; Fe_2O_3, 8.65 %; MgO, 5.67 %; CaO, 2.60 %; K_2O, 2.38 %; and Na_2O, 0.18 %. Before use, the raw palygorskite mineral was rolled on a three-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.

A total of 192 1-day-old Arbor Acres broiler chicks obtained from a commercial hatchery were allocated to four dietary treatments consisting of six replicates with eight chicks per replicate (four females and four males/replicate). Birds in the four treatments were given a basal diet supplemented with 0, 5, 10, and 20 g/kg palygorskite for 42 days, respectively. The basal diet was formulated according to the NRC [25]. The composition and nutrient content of basal diet are given in Table 1. Birds had free access to mash feed and water in three-level cages (120 cm × 60 cm × 50 cm; 0.09 m^2 per chick) in a temperature-controlled room with continuous lighting. The temperature of the room was maintained at 32 to 34 °C for the first 3 days and then reduced by 2 to 3 °C per week to a final temperature of 20 °C. At 42 day, birds were weighed by replicate (cage) after feed deprivation for 12 h and feed intake was recorded by cage to calculate average daily feed intake (ADFI), average daily gain (ADG), and feed/gain ratio (F/G). Birds that died during the experiment were weighed, and the data were included in the calculation of F/G.

Sample Collection

At 42 day of experiment, 24 male birds (one bird per replicate) were selected and weighed after feed deprivation for 12 h, which were subsequently killed by cervical dislocation. The pectoralis major muscle and thigh samples (iliotibialis lateralis, the area close to the wing) were immediately excised and stored at 4 °C for subsequent meat quality determination. The right pectoralis major muscle and thigh subsamples were used to measure meat color, pH values, and drip loss. The left pectoralis major muscle and thigh subsamples were used to determine cooking loss (weight loss during cooking) and shear force (only for pectoralis major muscle). The rest of the left pectoralis major muscle and thigh were immediately frozen and stored at −20 °C for the further analysis of antioxidant parameters and mineral elements contents.

Meat Quality Assay

The pH values were measured at 45 min and 24 h postmortem at a 1-cm depth using a pH meter (HI9125, HANNA Instruments, Italy) as described by Wang et al. [26] and Wu et al. [27]. Meat color was determined at 45 min after slaughter by a colorimeter (Minolta CR-400, Konica

Table 1 Composition and nutrient level of basal diet (g/kg, as fed basis unless otherwise stated)

Items	1–21 days	22–42 days
Ingredients		
Corn	576.1	622.7
Soybean meal	310	230
Corn gluten meal	32.9	60
Soybean oil	31.1	40
Limestone	12	14
Dicalcium phosphate	20	16
L-Lysine	3.4	3.5
DL-Methionine	1.5	0.8
Sodium chloride	3	3
Premix[a]	10	10
Calculated nutrient levels[b]		
Apparent metabolizable energy (MJ/kg)	12.56	13.19
Crude protein	211	196
Calcium	10.00	9.50
Available phosphorus	4.60	3.90
Lysine	12.00	10.50
Methionine	5.00	4.20
Methionine + cystine	8.50	7.60
Analyzed composition[c]		
Crude protein	208	192
Ash	57.2	56.5

[a] Premix provided per kilogram of diet: vitamin A (transretinyl acetate), 10,000 IU; vitamin D_3 (cholecalciferol), 3000 IU; vitamin E (all-rac-α-tocopherol), 24 IU; menadione, 1.3 mg; thiamin, 2.2 mg; riboflavin, 8 mg; nicotinamide, 40 mg; choline chloride, 400 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 sulfate), 8.0 mg; Mn (from manganese sulfate), 110 mg; Zn (from zinc oxide), 60 mg; I (from calcium iodate), 1.1 mg; and Se (from sodium selenite), 0.3 mg

[b] The nutrient levels were as fed basis

[c] Values based on analysis of triplicate samples of diets

Minolta, Tokyo, Japan) using the CIELAB system (L^* = lightness; a^* = redness; b^* = yellowness). The 24-h drip loss was measured postmortem according to the methods of Christensen [28] and Wang et al. [26]. In detail, muscles trimmed of adjacent fat and connective tissues were weighed, hung in a plastic bag, sealed, and then stored at 4 °C for 24 h. The meat was weighed again after removal from the plastic bag to determine drip loss that was calculated as gram of weight loss during 24 h per kilogram initial muscle weight. Cooking loss was measured 24 h after slaughter according to the method of Wang et al. [26] with minor modifications. Briefly, muscle samples were dried on the surface using filter papers, weighed, placed into a sealed plastic bag, and heated in a water bath to an internal temperature of 75 °C which was maintained for 20 min. The samples were then weighed again after cooling to room temperature. The cooking loss was calculated as gram of weight loss during cooking per kilogram muscle weight. Shear force was determined using a C-LM3 texture analyzer (Northeast Agricultural University, Haerbin, Heilongjiang, P. R. China) after meat was cooked to an internal temperature of 75 °C and then cooled to room temperature as described [29].

Muscular Antioxidant Determination

Approximately 0.3 g of muscle samples was homogenized (1:4, *w*/*v*) with ice-cold 154 mmol/L sodium chloride solution using an Ultra-Turrax homogenizer (Tekmar Co., Cincinnati, OH), and then centrifuged at 4550*g* for 15 min at 4 °C. The supernatant was used for assaying the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and ferric reducing-antioxidant power (FRAP), and the concentrations of malondialdehyde (MDA) and total protein using colorimetric kits with a 1200 UV spectrophotometer (Mapada Instruments Co., Ltd., Shanghai, China) according to the instructions of the commercial kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, Jiangsu, P. R. China). SOD activity was measured by xanthine oxidase method [30]. One unit of SOD activity was defined as the amount of enzyme per milligram protein of muscle that would produce 50 % inhibition of the rate of nitrite production at 37 °C. The methodology used in determining GSH-Px activity was the dithio-nitro benzene method described by Hafeman et al. [31], and one U of GSH-Px activity was defined as the amount of enzyme per milligram protein that would catalyze the conversion of 1 μmol/L of reduced glutathione to oxidized glutathione at 37 °C in 5 min. The FRAP assay was carried out according to the procedure of Benzie and Strain [32]. One unit of FRAP was defined as the amount of enzyme per milligram protein that increased the absorbance by 0.01 at 37 °C in 1 min. MDA content was measured following the thiobarbituric acid method described by Placer et al. [33]. All results were normalized against total protein concentration in each sample for inter-sample comparison. Total protein concentration was determined according to the method described by Bradford [34] using bovine serum albumin as the standard protein.

Determination of Mineral Element

The contents of magnesium (Mg), manganese (Mn), zinc (Zn), copper (Cu), Pb, Cd, and chrome (Cr) in the diets and muscle samples were measured according to the method of Tang et al. [35]. In detail, approximately 0.5 g feed or 2.0 g of muscle sample was weighed into a glass digestion tube, mixed with 10 mL of a mixture acid of nitric acid and perchloric acid

(4:1, *v*/*v*) at room temperature for 12 h and then digested on a heating block (LabTech DigiBlock Digester, EHD36, Labtech Co., Ltd., P. R. China) to obtain a clear digested solution. The procedure of digestion was given in the following: 90 °C for 30 min, 120 °C for 30 min, 160 °C for 120 min, and 180 °C for 210 min. Digested solutions were then diluted with ultra-pure water to a final volume of 25 mL. The final solutions were analyzed for mineral elements contents by inductively coupled plasma mass spectrometry (Optimal 2100DV, PerkinElmer-Sciex, Norwalk, NY, USA). The operating conditions were the following: power, 1300 W; plasma gas flow rate, 12 L/min; auxiliary gas flow rate, 0.2 L/min; nebulizer gas flow rate, 0.55 L/min; sample flow rate, 1.5 mL/min; and sample uptake rate, 1.0 mL/min. Validation of the mineral analysis was conducted using a certified bovine liver powder (GBW (E) 080193; National Institute of Standards and Technology, Beijing, P. R. China) as a standard reference material. Analyzed mineral element contents in the diets and muscles are presented in Table 2 (Cr and Cd were undetectable in the muscles and therefore were not presented in the table).

Statistical Analysis

Data was analyzed by one-way analysis of variance (ANOVA) using SPSS statistical software (Ver.16.0 for windows, SPSS Inc., Chicago, IL, USA). Differences among treatments were examined using Duncan's multiple range tests. The differences were considered to be significant at $P < 0.05$. Data were presented as means and their pooled standard errors.

Table 2 Analyzed mineral elements contents in the diets

Items[a, b]	Palygorskite (g/kg)			
	0	5	10	20
1–21 days				
Zn	116.3 ± 10.3	106.8 ± 5.9	103.6 ± 4.2	102.5 ± 0.2
Mn	153.0 ± 5.2	152.4 ± 1.3	162.6 ± 3.1	152.8 ± 4.1
Mg	2133 ± 30	2224 ± 41	2379 ± 3	2479 ± 51
Cu	19.6 ± 0.4	20.6 ± 0.4	21.8 ± 0.6	18.6 ± 0.2
Pb	1.69 ± 0.12	1.62 ± 0.17	1.57 ± 0.07	1.52 ± 0.19
22–42 days				
Zn	90.6 ± 8.6	91.9 ± 3.2	81.5 ± 1.2	81.5 ± 3.1
Mn	111.8 ± 0.5	116.1 ± 0.4	112.0 ± 3.9	107.9 ± 0.8
Mg	1877 ± 32	1941 ± 31	2031 ± 53	2143 ± 7
Cu	14.8 ± 0.5	14.1 ± 0.4	13.7 ± 0.2	13.4 ± 0.2
Pb	1.73 ± 0.12	1.64 ± 0.05	1.59 ± 0.12	1.53 ± 0.01

[a] Values based on analysis of triplicate samples of diets

[b] Means and standard error were presented

Results

Growth Performance

Compared with the control group (Table 3), neither 5 g/kg nor 10 g/kg palygorskite inclusion affected the growth performance of broilers during the 42-day study ($P > 0.05$). In contrast, the highest level of palygorskite supplementation at the level of 20 g/kg increased F/G of broilers ($P < 0.001$). However, ADG and ADFI of broilers were not altered by 20 g/kg palygorskite inclusion, though birds fed this diet (20 g/kg palygorskite) exhibited numerically reduced ADG and increased ADFI ($P > 0.05$).

Meat Quality

Birds fed diets (Table 4) supplemented with palygorskite exhibited similar pH value, drip loss, shear force, or cooking loss to those given basal diet at 42 days of age ($P > 0.05$). Similarly, compared with the control group, meat color was not altered by the inclusion of 5 g/kg palygorskite ($P > 0.05$). However, yellowness ($P < 0.001$) and redness ($P = 0.003$) of breast muscle and yellowness of leg muscle ($P = 0.001$) were decreased by palygorskite supplementation at the levels of 10 g/kg and especially 20 g/kg. Additionally, redness of leg muscle was also reduced by the inclusion of 20 g/kg palygorskite ($P = 0.009$). However, palygorskite supplementation did not change lightness value of meat ($P > 0.05$).

Muscular Oxidative Status

Compared with control group (Table 5), palygorskite supplementation had no effect on the activities of muscular SOD, GSH-Px, or FRAP at 42 days of experiment ($P > 0.05$). Likewise, the supplementation of 5 and 10 g/kg palygorskite did not alter muscular MDA concentration ($P > 0.05$). MDA accumulation in the breast muscle was significantly increased by the supplementation of 20 g/kg palygorskite ($P < 0.001$),

Table 3 Growth performance of broilers from 1 to 42 days

Items	Palygorskite (g/kg)				SEM	*P* value
	0	5	10	20		
ADG (g/d)	50.1	50.8	50.9	47.1	0.9	0.118
ADFI (g/d)	89.0	89.5	91.8	92.6	0.6	0.508
F:G (g:g)	1.78b	1.76b	1.80b	1.97a	0.02	$P < 0.001$

Means within a row with different letters (a–b) are different at $P < 0.05$

ADG average daily gain, *ADFI* average daily feed intake, *F:G* feed/gain ratio, *SEM* total standard error of means ($n = 6$)

Table 4 Meat quality of broilers at 42 days of age

Items	Palygorskite (g/kg)				SEM	*P* value
	0	5	10	20		
Breast						
$pH_{45\ min}$	6.49	6.54	6.52	6.44	0.04	0.892
$pH_{24\ h}$	5.87	5.94	5.88	5.89	0.03	0.888
Drip loss (g/kg)	47.7	55.9	58.1	38.9	0.1	0.351
Cooking loss (g/kg)	214	212	219	206	5	0.835
Shear force (kg)	2.36	2.30	2.34	2.23	0.04	0.606
Lightness	46.4	46.5	47.1	47.3	0.6	0.949
Redness	3.51a	3.17a	2.70b	2.13c	0.12	0.003
Yellowness	12.3a	11.7ab	10.2bc	8.6c	0.4	$P < 0.001$
Thigh						
$pH_{45\ min}$	6.53	6.59	6.57	6.59	0.04	0.959
$pH_{24\ h}$	6.16	6.24	6.14	6.16	0.03	0.993
Drip loss (g/kg)	108	117	104	111	8	0.887
Cooking loss (g/kg)	227	207	220	222	8	0.859
Lightness	51.9	53.3	53.7	53.1	0.5	0.639
Redness	9.43a	9.40a	8.36ab	7.54c	0.26	0.009
Yellowness	16.0a	15.4a	12.6b	11.5b	0.5	0.001

Means within a row with different letters (a–c) are different at $P < 0.05$

SEM total standard error of means ($n = 6$)

whereas the similar effect was not observed for MDA content in the thigh ($P > 0.05$).

Mineral Elements Contents

Palygorskite supplementation (Table 6) did not alter muscular (breast and thigh muscles) Zn, Mn, and Mg accumulations of broilers at 42 days of age ($P > 0.05$). Likewise, Cu content in the thigh was also similar among groups ($P > 0.05$). Compared with the control group, the inclusion of palygorskite at either 10 or 20 g/kg significantly decreased Pb accumulation in the breast ($P = 0.001$) or thigh ($P = 0.045$) and Cu accumulation in the breast ($P = 0.022$). However, this effect was not observed when the inclusion level of palygorskite was 5 g/kg ($P > 0.05$), and there were no significant differences in muscular mineral elements retention between 10 and 20 g/kg group ($P > 0.05$).

Table 5 Muscular antioxidant capacity of broilers at 42 days of age

Items	Palygorskite (g/kg)				SEM	*P* value
	0	5	10	20		
Breast						
FRAP (U/mg protein)	0.114	0.121	0.122	0.112	0.009	0.978
MDA (nmol/mg protein)	0.336b	0.308b	0.307b	0.767a	0.056	$P < 0.001$
SOD (U/mg protein)	24.6	24.0	25.4	24.4	0.5	0.785
GSH-Px (U/mg protein)	19.4	21.1	21.5	17.3	1.2	0.675
Thigh						
FRAP (U/mg protein)	0.050	0.055	0.057	0.045	0.004	0.723
MDA (nmol/mg protein)	1.99	1.94	1.83	2.09	0.15	0.949
SOD (U/mg protein)	17.1	17.4	18.7	17.0	0.5	0.626
GSH-Px (U/mg protein)	17.1	18.6	19.5	17.9	0.9	0.765

Means within a row with different letters (a–b) are different at $P < 0.05$

SOD superoxide dismutase, *GSH-Px* glutathione peroxidase, *MDA* malonaldehyde, *FRAP* ferric reducing-antioxidant power, *SEM* total standard error of means ($n = 6$)

Table 6 Muscular mineral elements contents of broilers at 42 days of age (mg/kg)

Items	Palygorskite (g/kg)				SEM	*P* value
	0	5	10	20		
Breast						
Zn	8.09	8.47	8.55	8.04	0.6	0.669
Mn	0.16	0.15	0.13	0.12	0.01	0.094
Mg	361	359	353	341	4	0.281
Cu	0.44a	0.42a	0.35b	0.34b	0.02	0.022
Pb	0.208a	0.205a	0.193b	0.184b	0.003	0.001
Thigh						
Zn	16.4	18	17.6	16.5	0.8	0.876
Mn	0.14	0.15	0.14	0.13	0.02	0.353
Mg	251	241	258	249	3	0.196
Cu	0.56	0.58	0.46	0.45	0.03	0.263
Pb	0.201a	0.186ab	0.177b	0.172b	0.004	0.045

Means within a row with different letters (a–b) are different at $P < 0.05$

SEM total standard error of means ($n = 6$)

Discussion

In this study, 5 and 10 g/kg palygorskite inclusion had no effect on the growth performance of broilers, but the highest level of palygorskite supplementation (20 g/kg) impaired growth performance as evidenced by increased F/G, indicating that the effect of palygorskite inclusion on the growth performance of broilers in the present study was closely associated with its dosage. The increased F/G may be due to the simultaneously reduced ADG and increased ADFI. Also, it is interesting to note that no parallel increasing trend among daily weight gain and feed intake of broilers fed diet supplemented with 20 g/kg palygorskite was found in the current study. This may be firstly associated with the nutrition dilution aroused by palygorskite supplementation. Also, palygorskite that possesses the binding potential for nutrients including pigments and trace mineral elements was postulated to account for this phenomenon [11, 23]. The results regarding growth performance in this study were partially in agreement with the results of Pappas et al. [4] who reported that dietary 1.0 % (10 g/kg) palygorskite supplementation used as a pellet binder did not alter the growth performance of broilers during a 6-week study. In contrast, Qiao et al. [36] recently reported that neither 2.0 % natural palygorskite nor 2.0 % heat-modified palygorskite supplementation changed feed intake and feed conversion ratio of laying hens. In the weaned piglets, the supplementation of 2 g/kg palygorskite improved feed conversion efficiency whereas a higher level of palygorskite inclusion at 3 g/kg did not change growth performance [3]. Animal species, dosage, age, and palygorskite variation would account for these discrepancies.

Vegetable oils, like poultry feed, contain numerous pigments including chlorophyll, carotenoids, xanthophylls, and their derivatives, which are subsequently removed to give the oil a color that is acceptable to the consumer during the edible oil manufacture process [37]. Activated clays including palygorskite, sepiolite, and bentonite are the popular adsorbents for decolorization of edible oil due to their less expensive price than activated carbon and high specific area and adsorption capacity [13, 38–41]. In poultry, Blair et al. [9] noted that laying hens fed diets supplemented with spent bleaching clay, a bentonite product from canola oil refining, had reduced yolk color. Similar results were also observed by Hashemipour et al. [10] with sodium bentonite supplementation in laying hens diets. This study found that palygorskite inclusion can reduce yellowness and redness of meat, and this effect became more pronounced with the increasing dosage of palygorskite (from 10 to 20 g/kg). The results obtained in the current study were consistent with findings of Chalvatzi et al. [11] in which laying hens given diets supplemented with 1.0 % palygorskite for 24 weeks produced eggs with significantly lighter eggshell and yolk color. Most of the variation in meat color of normal meat quality can be explained by the pigment content, myoglobin forms, and internal reflectance [6], which in turn suggested that the reduced meat color (yellowness and redness) observed in this study may be due to the affinity between palygorskite and pigments [13, 41].

Free radicals that mainly consist of radical oxygen species and radical nitrogen species are produced during normal metabolism in cells, and oxidative stress is an imbalance between the efficiency of the antioxidant defense and the free radical generation [16, 42]. To protect organelles and cellular components against free radical-associated damage, cells have developed several antioxidant systems, including enzymatic and non-enzymatic antioxidant systems. Natural pigments are usually classified as constituents of non-enzymatic antioxidant system [43, 44]. Carotenoids, such as β-carotene and lutein, are a major class of lipophilic colorants and free radical scavengers [45]. In this study, the supplementation of 20 g/kg palygorskite increased MDA accumulation in the breast of broilers. MDA is the main end product of lipid peroxidation by radical oxygen species, and increased MDA accumulation is an important indication of lipid peroxidation [46]. The increased MDA accumulation, consistent with the simultaneously reduced yellowness and redness value of meat, indicated that the highest level of palygorskite supplementation decreased the antioxidant capacity of breast muscle, which may be due to the decreased muscular pigment retention since several nature pigments possess antioxidant capacity [14, 15, 45]. It is interesting to note that the highest level of palygorskite supplementation only altered the oxidative status of breast muscle but not thigh muscle despite the fact that yellowness and redness values of thigh were still reduced by

palygorskite supplementation. This difference may result from the different antioxidant abilities of measured muscles. Alasnier et al. [47] reported that chicken thigh muscles contained two to four times less thiobarbituric acid reactive substances when stored at 4 °C between 1 and 14-day post-mortem. Also, the difference in soluble selenium content between breast and thigh muscles as demonstrated by Daun and Åkesson [48] may also contribute to this preference.

Studies have demonstrated that palygorskite can be used as an absorbent to remove Cu and Pb from aqueous solution and soils due to its high specific area and adsorption capacity [20–22, 49]. However, there were no studies regarding palygorskite supplementation on the mineral element accumulations in poultry. The supplementation of another clay, montmorillonite, has been proven to reduce Cd and Pb retention in the tissues of aquatic animals [50, 51]. In the present study, palygorskite supplementation that exceeded 5 g/kg also significantly decreased muscular Cu and Pb accumulation, indicating that palygorskite could be also used to decrease Cu and Pb retention in vivo and this effect was associated with its dosage. However, Zhang et al. [23] have recently found that the inclusion of 2 % palygorskite decreased the retention of Cd rather than Pb or Cu in the muscle of blunt snout bream (*M. amblycephala*). This discrepancy is likely to result from animal species and different contents of mineral elements in the diet. Also, it is meaningful to note that the capacity of palygorskite to reduce Pb accumulation was more pronounced than Cu. Similarly, Potgieter et al. [21] also reported that the capacity to adsorb metals by palygorskite from the single-metal solutions in vitro was in the following order: Pb > Cu.

In conclusion, the growth performance, meat color (redness and yellowness), and muscular oxidative status would gradually decrease with the increasing level of palygorskite supplementation especially at the level of 20 g/kg. However, high levels of palygorskite that exceeded 5 g/kg (10 or 20 g/kg) can alter mineral element contents in muscles, which was demonstrated by the decreased muscular Cu and Pb concentrations.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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