

Palygorskite combined probiotics improve the laying performance, hatching performance, egg quality, plasma antioxidative status, and immune response of broiler breeders

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ABSTRACT

This experiment was conducted to investigate the effects of different supplementation levels of palygorskite and probiotics complex (Pal-Pro) on laying performance, hatching performance, egg quality, plasma antioxidative status, and immune response of broiler breeders. A total of 432 Xuefeng black-bone chickens (42-week-old) were randomly assigned into four treatments with six replicates of 18 hens. The broiler breeders were fed the basal diet supplemented with 0, 250, 500, and 750 mg/kg Pal-Pro for 8 weeks. The Pal-Pro supplementation linearly or quadratically increased ($p < .05$) laying rate, fertilisation rate, hatchability of fertile eggs, hatch hatched chicks rate, eggshell thickness, yolk index, total antioxidant capacity, activities of total superoxide dismutase, catalase, glutathione peroxidase, and contents of immunoglobulins, interleukin-2, and tumour necrosis factor- α in plasma. With Pal-Pro supplementation increased, feed conversion ratio during 5–8 and 1–8 week, embryo mortality, malondialdehyde linearly decreased ($p < .05$). This finding suggested that dietary supplementation with 750 mg/kg Pal-Pro administration could improve the antioxidative status and immunoglobulins contents in plasma of broiler breeders, which may contribute to an increase in broiler's laying rate, hatching performance, egg quality, such as eggshell thickness and yolk index.

HIGHLIGHTS

- Dietary supplementation of Pal-Pro decreased the feed conversion ratio and embryo mortality of broiler breeders.
- Dietary supplementation of Pal-Pro improved the laying rate, fertility, hatchability of fertile eggs, and yolk index of broiler breeders.
- Dietary supplementation of Pal-Pro enhanced the antioxidant capacity and the level of immunoglobulins in the plasma.

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Introduction

Feeds are frequently co-contaminated with the mycotoxins through concentration complied with the regulations of the Food and Agriculture Organisation of the United Nations (Streit et al. 2012; Kemboi et al. 2020). However, many studies showed that poultry exposed to mycotoxin for a long time could result in slow growth, metabolic and reproductive disorders, and immunosuppression, even death (Vesonder and Wu 1998; Swamy et al. 2004; Weaver et al. 2020). There are also have negative effects on pigs (Wu et al. 2021), ducks (Arak et al. 2020), ruminants (Mostrom and Jacobsen 2020), fishes (Bobadilla-Carrillo et al. 2020), and rabbits (Liu et al. 2020). At the same time,

China has completely prohibited the addition of antibiotics as growth promoters in animal feed since 1 July 2020 (<http://www.moa.gov.cn/gk/>, accessed on January 6th, 2020). Therefore, it is necessary that finding safe and effective feed additives to reduce the harm of mycotoxins and maintain animal health, which is a challenge for the efficient production of broiler breeders.

Palygorskite (Pal), a hydrated magnesium-rich silicate clay mineral, has the characteristics of large specific surface area, plentiful pores, and high adsorption capacity (Wang and Wang 2016). It has been reported that supplementation of Pal in the diets could ameliorate mycotoxins' toxic effects on pigs (Zhang et al.

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Table 1. Composition and nutrient levels of the experimental diets (dry matter basis).

Item	Content
Ingredient, %, unless otherwise indicated	
Corn	65.00
Soybean meal	25.00
Limestone	6.50
CaHPO ₄	1.00
Premix ^a	2.50
Total	100.00
Calculated nutrient composition	
Metabolisable energy, MJ/kg	11.30
Crude protein	16.78
Lys	0.78
Met + Cys	0.55
Met	0.27
Ca	3.50
Available P	0.30
Aflatoxin B ₁ , µg/kg	3.44
Zearalenone, µg/kg	27.30

^aPremix provided per kilogram of diet: vitamin A 9000 IU, vitamin D3 2500 IU, vitamin E 20 IU, vitamin B2 5.5 mg, vitamin K3 1 mg, Cu 8 mg, Fe (as ferrous sulfate) 75 mg, Zn (as zinc sulfate) 60 mg, Mn (as manganese sulfate) 75 mg, Se (as sodium selenite) 0.30 mg, I (as potassium iodide) 1 mg, Ca 6.75 g, P 1.125 g, NaCl 3.50 g, folic acid 0.75 mg, nicotinamide 30 mg, pantothenic acid 9 mg.

2013) and broilers (Cheng et al. 2018). Besides, other researchers found that dietary supplementation with Pal could improve growth performance, antioxidant capacity, immunity, and intestinal health of animals (Schell et al. 1993; Cheng et al. 2016). Probiotics (Pro) are living microorganisms that bring health benefits to the host. Plenty of studies on poultry demonstrated that a diet supplemented with Pro could mitigate and decrease mycotoxins toxic effects (Markowiak et al. 2019; Chang et al. 2020), and enhance animal's health and performance through regulating the balance of bacteria in the intestinal tract (Chen et al. 2020; Rodrigues et al. 2020).

Xuefeng black-bone chicken, a valuable race native breed in the Hunan province of China, is a dual-purpose chicken bred for its meat and eggs, which is loved by people due to its high nutritional value. Our previous studies on laying hens found that added of *Bacillus subtilis* and montmorillonite independently or in combinations in the diet can improve egg quality, antioxidant and immune status (Chen et al. 2019, 2020), there is also a similar effect about the mixture of *Bacillus subtilis* and essential oils (Liu et al. 2020). It is a trend that combined the use of multiple additives to improve animal health in the future, there are seldom studies on the combination of Pal and Pro in broiler breeders. We hypothesised that add Pal-Pro to broilers' diets would affect production performance, plasma oxidative status, and immune response of broiler breeders. Therefore, the present study was conducted to investigate different levels of Pal-Pro supplementation on the laying performance, reproductive

performance, egg quality, plasma oxidative status, and immune response of broiler breeders, which provide a theoretical reference for the rational application of Pal-Pro in broiler breeders.

Materials and methods

All the birds and the experimental protocols were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Hunan, China.

The Pal-Pro

The Pal-Pro final product, SINIPRO MAX, was provided by Jiangsu Sinitic Biological Technology Co., Ltd. (Jiangsu, China) and was composed of palygorskite, *Bacillus subtilis* ($\geq 0.5 \times 10^9$ CFU/kg), and *Bacillus licheniformis* ($\geq 0.5 \times 10^9$ CFU/kg).

Chickens, experimental design, and management

Xuefeng black-bone chicken was obtained from Hunan Yunfeifeng Agricultural Commercial Company (Hunan, China). A total of 432 healthy Xuefeng black-bone chickens at 42-week of age with similar weight were randomly and equally divided into four treatments with six replicates of 18 hens. The dietary treatments were as follows: (1) basal diet; (2) basal diet + 250 mg/kg Pal-Pro; (3) basal diet + 500 mg/kg Pal-Pro; (4) basal diet + 750 mg/kg Pal-Pro. The composition and ingredients of the diets showed in Table 1. Representative diets were taken before the experiment start for determined the aflatoxin B₁ and zearalenone concentrations by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Romer Labs Co., Ltd., Beijing, China), and also shown in Table 1. The experimental diets were formulated to meet nutrient requirements according to the China Agricultural Standard (NY/T 33-2004) (Wen et al. 2004).

This feeding experiment was conducted from July to September 2020 at the original breeding farm of Hunan Yunfeifeng Agricultural Commercial Company. Assigned to experimental each replicate with 6 cages and 3 hens were raised in a cage (38 × 28 × 36 cm; length × width × height). After 7 days of adaptation, all hens were fed the assigned experimental diets for 56 days. The hens were fed twice a day (06:30 and 14:30) and given *ad libitum* access to water throughout the experiment. The temperature and relative humidity in the chicken coop was 27.55 ± 2.35 °C and

$78.65 \pm 3.68\%$ (mean \pm SD), respectively. The lighting regimen used was a 16 h light and 8 h darkness cycle throughout the entire experimental period.

Laying and hatching performance

During the experiment period, the number of eggs, egg weight, and feed intake were recorded weekly of each replicate, and eggs were graded as qualified and unqualified (the unqualified included broken eggs, no shell eggs, excessively large or small eggs, and misshapen eggs) to calculate the laying rate, qualified egg rate, and feed conversion ratio (total feed intake/total egg weight in each replicate). To ensure data credibility, mortality and health status were visually recorded daily. Artificial insemination is performed on the broiler breeders at 14:00 every five days. About 2.5 mL semen from 10 cocks was collected and mixed with the same amount of diluent, and then fertilised 35 μ L per hen as described by Liu et al. (2019). The number of infertile eggs, dead embryos, and weak hatched chicks were recorded weekly by replicate to calculate fertilisation rate, embryo mortality, hatchability of fertile eggs, and health hatched chickens rate.

Egg quality

During the experiment period, the egg quality was measured on three eggs collected randomly from each replicate at 56 d. These eggs were kept at 4 °C and analysed within 2 days after collection to determine egg quality indexes. The yolk and albumen were separated manually by an egg separator and then weighted the yolk. The length and width of the egg and the width and height of yolk were measured by an electronic digital caliper (SH14100025, Shenhan, Shanghai, China) to calculate the egg shape index (egg width/egg length) and yolk index (yolk height/yolk width). Haugh unit was measured by an egg analyser (EA-01; Orka Food Technology, Ramat HaSharon, Israel). Eggshell thickness was the mean of measurements at three locations on the large end, equatorial region, and sharp end of eggs using a digital micrometer (NFN380; FHK, Japan). Eggshell-breaking strength was measured by egg force reader (EFR-01; Orka Food Technology).

Blood sample collection

At the end of the experiment, 12 hens (two hens per replicate) were randomly selected from each treatment after fasting for 12 h. Blood samples (5 mL) were

obtained *via* the sub-wing vein using the disposable needle, then immediately transferred into the vacutainer tube. Blood samples were placed at room temperature for 1 h, subsequently centrifuged at $3000 \times g$ for 10 min to separate plasma, and plasma was stored in 1.5 mL Eppendorf tube at -80°C for further analysis.

Assay of antioxidant indexes and immune indexes in plasma

The antioxidant indexes include total antioxidant capacity (T-AOC), total superoxide dismutase (T-SOD), catalase (CAT), malondialdehyde (MDA), and glutathione peroxidase (GSH-Px). The immune indexes include immunoglobulins A (IgA), immunoglobulins G (IgG), immunoglobulins M (IgM), Interleukin-2 (IL-2), and tumour necrosis factor- α (TNF- α). Above indexes were determined using chicken enzyme-linked immunosorbent assay (ELISA) Kits (ZC-51990; ZC-51690; ZC-51753; ZC-51675; ZC-51747; ZC-51833; ZC-51835; ZC-51839; ZC-51660; ZC-51975; ZCIBIO Technology Co., Ltd., Shanghai, China) with microplate reader according to the instructions of the manufacturer and all standards were tested in duplicate.

Determination of endotoxin concentration

At 56 days, two eggs and two plasma samples were randomly collected from each replicate. Egg yolk and albumen were blended with a tissue homogeniser. Protein concentration and endotoxin (ET) concentration were determined with the BCA Kit (CW0014S; Beijing ComWin Biotech Co., Ltd., Beijing, China) and the Chicken ET ELISA Kit (E-75003; Shanghai Enzyme-linked Biotechnology Co., Ltd., Shanghai, China), respectively. The measurement operation procedure was carried out according to the instructions.

Statistical analyses

All data were analysed by one-way analysis of variance (ANOVA) using SPSS 22.0 statistical software (SPSS Institute Inc., Chicago, Illinois), and the replicate was used as the experimental unit. Orthogonal polynomial comparisons were used to test the linear and quadratic responses to the Pal-Pro levels. $p < .05$ was considered statistically significant.

Table 2. Effects of Pal-Pro on laying performance of broiler breeders^a.

Item	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
1–4 week							
Qualified egg rate, %	96.94	96.82	97.89	97.61	0.234	.220	.488
Egg weight, g	49.03	47.85	48.18	49.05	0.253	.559	.052
Laying rate, %	49.09	50.33	50.53	52.89	0.586	.034	.618
ADFI, g/d	84.36	84.56	84.88	84.97	0.556	.705	.902
FCR, g of feed/g of egg	3.51	3.52	3.50	3.35	0.037	.290	.167
5–8 week							
Qualified egg rate, %	96.31	96.39	97.10	96.30	0.372	.788	.793
Egg weight, g	50.05	49.64	49.74	50.37	0.179	.888	.143
Laying rate, %	48.28	51.85	54.02	54.86	0.849	.004	.371
ADFI, g/d	84.39	86.56	88.20	87.47	0.681	.054	.811
FCR, g of feed/g of egg	3.51	3.37	3.29	3.24	0.043	.018	.723
1–8 week							
Qualified egg rate, %	96.62	96.61	97.51	96.97	0.219	.357	.896
Egg weight, g	49.53	48.76	48.98	49.70	0.206	.796	.078
Laying rate, %	48.68	51.07	52.23	53.85	0.650	.004	.733
ADFI, g/d	84.35	85.53	86.47	86.18	0.501	.140	.934
FCR, g of feed/g of egg	3.51	3.44	3.39	3.29	0.036	.048	.360

ADFI: average daily feed intake; FCR: feed conversion ratio; Pal-Pro: palygorskite and probiotics complex.

^aData are means of six replicates with 18 hens per treatment.

Results

Laying performance

The effects of different levels of Pal-Pro in diets on the laying performance of broiler breeders were shown in Table 2. The Pal-Pro treatments increased (linear, $p < .05$) laying rate at the 1–4 week, and decreased (linear, $p < .05$) feed conversion ratio during the 5–8 and 1–8 week. However, there were no differences in qualified egg rate, egg weight, and average daily feed intake among four treatments in the entire period of the experiment.

Hatching performance

The hatching performance results were presented in Table 3. The Pal-Pro treatments increased (linear, $p < .05$) fertilisation rate, hatchability of fertile eggs, and health hatched chicks rate and decreased (linear, $p < .05$) embryo mortality.

Egg quality

As listed in Table 4. The results showed that the Pal-Pro treatments increased (linear or quadratic, $p < .05$) eggshell thickness and yolk index. However, there were no differences in the egg shape index, eggshell breaking strength, Haugh unit, and yolk percentage among the four treatments.

Plasma antioxidant capacity and immune response

The results of antioxidant capacity were presented in Table 5. As the supplement of Pal-Pro increased, the concentrations of T-AOC, T-SOD, CAT, and GSH-Px increased (linear, $p < .05$) and MDA content decreased (linear, $p < .05$). As shown in Table 6. The Pal-Pro treatments increased (linear or quadratic, $p < .05$) the concentrations of IgA and IgG. Meanwhile, the concentrations of IgM, IL-2, and TNF- α in plasma increased (linear, $p < .05$).

Endotoxin concentration of plasma and egg

As pretended in Table 7, The Pal-Pro treatments decreased (linear or quadratic, $p < .05$) the endotoxin concentration of plasma. There were no differences in endotoxin concentration of eggs among the four treatments.

Discussion

Xuefeng black-bone chicken is a famous native breed in the Hunan province of China, which favoured by people due to its high nutritional value. However, there are still problems in the Xuefeng black-bone chicken industry, such as long feeding periods and low laying rate which need some measure to solve (Liu et al. 2020; Xie et al. 2020). Previous studies revealed that Pal or Pro can mitigate the adverse effects of mycotoxins on production performance, and change intestinal morphology and barrier function of animals (Cheng et al. 2018; Chen et al. 2019; Śliżewska et al. 2019; Wu et al. 2019). In our study, these data

Table 3. Effects of Pal-Pro on hatching performance of broiler breeders^a.

Item	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
Fertilisation rate, %	84.76	90.91	89.23	91.98	0.825	.002	.196
Embryo mortality, %	5.97	4.58	3.97	3.67	0.301	.004	.295
Hatchability of fertile eggs, %	86.45	90.15	92.06	92.21	0.666	<.001	.085
Health hatched chicks rate, %	97.57	98.99	99.17	99.12	0.247	.023	.109

Pal-Pro: palygorskite and probiotics complex.

^aData are means of last 6 weeks all eggs of per treatment.

Table 4. Effects of Pal-Pro on egg quality of broiler breeders^a.

Item	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
Egg shape index	1.32	1.35	1.32	1.33	0.006	.563	.458
Eggshell breaking strength, kgf	3.72	3.73	3.83	3.76	0.096	.796	.982
Eggshell thickness, mm	0.29	0.35	0.34	0.32	0.005	<.001	<.001
Haugh unit	64.54	65.59	65.52	68.32	0.715	.122	.303
Yolk index	0.39	0.42	0.43	0.43	0.005	.002	.850
Yolk percentage, %	33.61	33.55	32.16	33.15	0.003	.289	.906

Pal-Pro: palygorskite and probiotics complex.

^aData are means of 18 eggs per treatment.

Table 5. Effects of Pal-Pro on plasma antioxidant capacity of broiler breeders^a.

Item	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
T-AOC, U/mL	9.94	15.40	16.13	18.03	0.455	<.001	.824
T-SOD, ng/mL	4.81	7.30	8.34	8.78	0.233	<.001	.577
CAT, pg/mL	568.32	759.81	801.53	844.10	15.936	<.001	.490
MDA, nmol/mL	13.02	9.69	8.99	8.17	0.275	<.001	.599
GSH-Px, ng/mL	75.00	119.31	133.17	143.37	3.892	<.001	.692

T-AOC: total antioxidant capacity; T-SOD: total superoxide dismutase; CAT: catalase; MDA: malondialdehyde; GSH-Px: glutathione peroxidase; Pal-Pro: palygorskite and probiotics complex.

^aData are means of six replicates with two hens per treatment.

Table 6. Effects of Pal-Pro on plasma immune indexes of broiler breeders^a.

Item	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
IgA, µg/mL	123.70	249.56	271.85	293.93	9.739	<.001	.002
IgG, µg/mL	924.23	1779.91	2058.19	2145.54	71.328	<.001	.035
IgM, µg/mL	443.15	765.74	791.64	939.74	26.809	<.001	.056
IL-2, pg/mL	88.09	200.52	229.65	257.38	9.577	<.001	.958
TNF-α, pg/mL	31.13	49.73	50.43	60.44	1.656	<.001	.191

IgA: immunoglobulins A; IgG: immunoglobulins G; IgM: immunoglobulins M; IL-2: Interleukin-2; TNF-α: tumour necrosis factor-α; Pal-Pro: palygorskite and probiotics complex.

^aData are means of 6 replicates with 2 hens per treatment.

Table 7. Effects of Pal-Pro on endotoxin level in egg and plasma of broiler breeders^a.

Sample	Dietary Pal-Pro concentration, mg/kg				SEM	p-Value	
	0	250	500	750		Linear	Quadratic
Egg, ng/L	111.91	91.64	94.10	95.03	3.313	.125	.115
Plasma, ng/L	169.59	126.47	131.64	135.39	4.386	.006	.003

Pal-Pro: palygorskite and probiotics complex.

^aData are means of six replicates with two hens or two eggs per treatment.

implied that added Pal-Pro to the diet increased laying rate and decreased FCR. The results were corroborated by Chalvatzi et al. (2016) and Zhang et al. (2019), who reported that dietary supplements with 0.5% of modified Pal or 7.5×10^8 CFU/kg Pro could improve production performance, decrease FCR and change the caecal microbiota composition of laying hens. Previous investigations have demonstrated that Pal or Pro not only gradually reduced the absorption of low-level mycotoxins and growth of pathogen in the intestine tract, but also increased the activities of digestive enzymes in the intestinal tract (Hosoi et al. 2000; Xia et al. 2004; Shannon et al. 2017; Li et al. 2018), thus increase the efficiency of absorbing nutrients and ultimately improve the laying performance of broiler breeders.

This experiment is the first time to study the effect of Pal-Pro on the hatching performance of broiler breeders. It is important to note that dietary Pal-Pro has a positive effect on fertilisation rate, embryo mortality, hatchability of fertile eggs, and health hatched chicks rate. Prazdnova et al. (2019) demonstrated that *Bacillus subtilis* increased fertilisation rate and reduced the number of embryo deaths during the first 7 days of incubation. And Mazanko et al. (2018) also reported that bacillus probiotic positively affected the hatchability of laying hens and sperm production of roosters. However, another research found that both multi-strain probiotic and single-strain probiotic did not affect the hatching performance of broiler breeders (Aalaei et al. 2018). The inconsistent results may be owing to the different supplementation dosages and species. And there were several studies revealed that lower hatchability and higher embryo mortality were

found when hens were fed mycotoxin-contaminated feed (Ebrahim et al. 2014), because mycotoxins could residual in eggs (Mojgani et al. 2020). Therefore, the improvement of hatching performance may be that dietary Pal-Pro reduced mycotoxins transferred to eggs and increased the level of reproductive hormones (Wang et al. 2017; Zhou et al. 2020). As researches on the effects of Pal and Pro on hatching performance of broiler breeders are very limited, further study is still necessary to investigate its effect on the animal.

Eggshell thickness, Haugh unit, and yolk index are important factors for the preservation and transportation of hatching eggs. Lots of studies reported that clay mineral or Pro can increased eggshell thickness through increased calcium solubility and absorption in the intestinal tract (Chalvatzi et al. 2014; Chen et al. 2019; Liu et al. 2019). In the present study, the data showed that yolk index and eggshell thickness increased. However, there were no differences in shape index, shell strength, and Haugh unit in Pal-Pro treatments. Similar results have also been documented by Denli et al. (2008) and Qu et al. (2018). The yolk index is mainly affected by the albumen and the moisture of yolk, the water in the albumen penetrates the yolk through the yolk membrane results in the yolk index decreased over time (Hidalgo et al. 1996; Wang et al. 2015). The increase of yolk index could be related to the antioxidant status in the yolk and albumen (Pappas et al. 2005).

Oxidative stress refers to the imbalance of oxidation and antioxidation in the body, and it could produce varieties of reactive oxygen species (ROS), such as hydroxyl free radicals and superoxide anions, which can damage the proteins, nucleic acids, and other biological macromolecules, and leading to tissue damage and tissue mitochondrial damage (Jimenez et al. 2019; Pisoschi et al. 2021). With the development of modern intensive farming, animals are prone to oxidative stress (Surai et al. 2019). Antioxidant enzymes serve as the first line to suppress oxidative damage. Zhou et al. (2014) reported that dietary supplementation with 1% the combination of zeolite and attapulgit significantly increased CAT activity and decreased the MDA content of broiler. Yu et al. (2019) demonstrated that dietary 100 mg/kg of *Enterococcus faecium* significantly increased CAT, GSH-Px, and T-AOC activities in the breast meat of 42 day-old broilers. Our previous research on laying hens found that *Bacillus subtilis* and montmorillonite supplementation on plasma T-AOC and the activity of CAT in the spleen have a significant interaction effect (Chen et al. 2019). This study

showed that the concentrates of T-AOC, GSH-Px, T-SOD, and CAT increased and MDA content decreased in plasma of broiler breeders by supplemented with Pal-Pro. Our data indicated that dietary Pal-Pro may offer benefits to the antioxidant defense system of broiler breeders. The result was in agreement with the results of previous studies in broiler chickens (Bai et al. 2016; Cheng et al. 2018; Khan et al. 2018). Studies showed that probiotics can improve the activity of antioxidant enzymes *in vivo* by regulating the expression of oxidation-related genes, such as nuclear factor erythroid 2-related factor 2 and haem oxygenase-1 (Bai et al. 2017). Therefore, it is possible to assume that dietary probiotics in diets could enhance the antioxidant capacity of chickens mainly due to its capacity for activating relative gene expression. Besides, it has been proved that probiotics could produce polysaccharides, which can scavenging free radical. Meanwhile, Pal improved antioxidant status due to that the rod-like crystal structure of it can reduce the production of lipid peroxides (Cervini-Silva et al. 2015) and Pal decreased the adverse effect of mycotoxins.

The plasma of immunoglobulin reflects the change of immune function. Both IL-2 and TNF- α are secreted by T-helper type 1 cytokines, and their content increases in a state of low-grade inflammation to stimulate the maturation and activity of immune cells (Woda et al. 2016). In the present study, diet supplement with Pal-Pro improved the concentrations of IgA, IgG, IgM, IL-2, and TNF- α . Chen et al. (2016) reported that dietary 10 g/kg palygorskite supplementation significantly increased the concentrations of IgM and sIgA, and tended to increase the IgG content of ileum. Zhen et al. (2019) found that IgG, IgA, and IL-2 in serum significantly improved when broilers diets were supplemented with 2% lactobacillus. It shows that both Pro and Pal are beneficial to immune function, which is similar to our study. Studies have shown that Pro influenced immune function in two ways. Firstly, Pro can be used as non-specific immune factors to stimulate host cells through themselves or cell wall components, thereby activating macrophages; secondly, Pro metabolites, similar to immune adjuvants, can activate natural killer cells and CD8⁺ T cells and induce T-lymphocyte, B-lymphocyte, and macrophages to produce cytokines (Rajput et al. 2013; Fathi et al. 2018; Lee et al. 2020). And Pal could increase the secretion of secretory immunoglobulin A and reduce the entry of pathogens and mycotoxins into the mucosal barrier to improving immune ability (Slamova et al. 2011; Schaumberger et al. 2014). As far as immunoglobulin contents are concerned, diet

supplements with Pal-Pro have shown a positive effect of immunity. However, excessive TNF- α content is harmful to immunity because it is a proinflammatory cytokine, which concentrations of TNF- α are beneficial to animals health need to be further explored.

Endotoxin exists widely in the animal body, and it can induce monocytes and macrophages to produce inflammatory factors, such as TNF- α , IL-1, and IL-6 (Cavaillon 2018). In this study, there is no effect on the endotoxin concentration in the eggs but the endotoxin concentration of plasma is reduced when diet supplement with Pal-Pro. There are no reports on the endotoxin concentration in eggs at present, It may provide a reference for the follow-up study about egg endotoxin. The Pal-Pro can effectively reduce the endotoxin concentration in plasma, which is similar to previous studies (Drucker et al. 1977; Deng et al. 2017). The possible mechanism is that the Pal or the Pro improves intestinal function and reduces intestinal endotoxin into the blood circulation (Cervini-Silva et al. 2015; Wang et al. 2019; Sun et al. 2020; Xu et al. 2020).

Conclusion

In conclusion, dietary Pal-Pro supplementation at a dosage of 750 mg/kg could enhance the antioxidant capability and immune response in plasma of broiler breeders, which may benefit to improve laying rate, hatching performance, egg quality, such as eggshell thickness and yolk index. This study showed that Pal-Pro could as a potential feed additive for broiler breeders.

Ethical approval

All animal research projects were sanctioned by the Hunan Agricultural University New Rural Development Research Institute Characteristics Industrial Base Project—Xuefeng Black-bone Chicken Special Industrial Base Construction Project (J16101). All the birds and experimental protocols in this study were approved by the Institutional Animal Care and Use Committee of Hunan Agricultural University, Hunan, China.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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