

# Dietary apple pectic oligosaccharide improves reproductive performance, antioxidant capacity, and ovary function of broiler breeders

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**ABSTRACT** Reproduction performance is one of the most important economic traits for the poultry industry. Intriguingly, apple pectic oligosaccharide (APO) could promote gastrointestinal function and immune function to improve performance; however, literature about APO on reproduction performance in breeders is limited. This study aimed to determine whether APO administration can improve reproduction performance and ovary function of broiler breeders with different egg laying rates. Two hundred and fifty six Arbor Acres broiler breeders (48-week-old) were used in a  $2 \times 2$  factorial design with 2 egg laying rates (average [AR] and low [LR]) and 2 dietary levels of APO (0 and 200 mg/kg APO). Results showed that the LR breeders presented higher egg weight but lower egg laying rate, qualified egg rate, and feed efficiency than the AR breeders ( $P_{\text{(laying)}} < 0.05$ ). Also, the LR breeders had decreased serum Anti-Müllerian hormone, leptin, and antioxidant enzyme (superoxide dismutase, total antioxidant capacity) levels than the AR breeders ( $P_{\text{(laying)}} \leq 0.05$ ). Dietary supplementation with APO improved egg weight, feed efficiency, as well as

egg albumen quality (higher albumen height and Haugh unit) ( $P_{\text{(APO)}} < 0.05$ ), and decreased the concentration of pro-inflammatory cytokine levels (interleukin [IL]-1 $\beta$ , IL-8) in serum ( $P_{\text{(APO)}} \leq 0.05$ ). The apoptosis rate and pro-apoptosis-related gene expression (*caspase 9* and *Bax*) in the ovary of LR breeders were higher, while the anti-apoptosis-related gene expression (*Bcl-2*, *PCNA*) was lower in LR compared with the AR breeders ( $P_{\text{(laying)}} < 0.05$ ). Dietary supplementation with APO decreased the *caspase 9* and *Bax* expression in LR breeders ( $P_{\text{(interaction)}} < 0.05$ ), and increased the *Bcl-2* and *PCNA* expression in the 2 breeders ( $P_{\text{(APO)}} < 0.05$ ). These findings indicate that breeders with a lower egg laying rate exhibit lower antioxidant capacity and high cell apoptosis in the ovary. Dietary supplementation with APO might improve albumen quality and antioxidant capacity, and decrease the inflammatory factors and ovary apoptosis-related genes expression to improve ovary function. Moreover, the effect of APO on decreasing ovarian pro-apoptosis-related gene expression was more pronounced in lower reproductive breeders.

**Key words:** broiler breeder, apple pectic oligosaccharide, reproduction performance, antioxidant capacity, ovary function

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## INTRODUCTION

The reproduction performance of broiler breeders plays an important role in the poultry industry. Ovary function is the main factor affecting the reproductive performance of poultry (Johnson, 2012). Several studies

have demonstrated that functional oligosaccharide (such as chitosan oligosaccharide, mannan-oligosaccharide, galacto-mannan-oligosaccharide) could improve ovary function and litter size to enhance reproductive performance in sows and rats (Duan et al., 2016; Wan et al., 2016; Xu et al., 2018). Pectic oligosaccharides consist of pectic disaccharide and trisaccharide (Gullnón et al., 2013; Liu et al., 2020), and have been shown to improve mineral absorption, and regulate lipid metabolism, immune function, antioxidant capacity, and intestinal health in mice, humans, and pigs (Li et al., 2010, 2013; Gullnón et al., 2013). Dietary supplementation with apple pectic oligosaccharide (APO) appears to improve growth performance, antioxidant capacity, microbiota

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composition, and intestinal mucosal morphology in piglets too (Chen et al., 2017; Mao et al., 2019). It is well-known that the intake of nutrients and functional additives affects the reproductive performance of humans and animals. Also, a previous study showed that APO treatment can increase the reproduction of rats (Liu et al., 2020). However, studies on the effect of APO on reproductive performance and ovary function in breeders are limited. Moreover, broiler breeders may present different reproductive performances, despite sharing the same genomic background, diet, and environment. Also, in our previous study, we found that the improving effect of dietary probiotics (*Enterococcus faecium*) in egg weight and microbiota enrichment were more pronounced in low reproductive breeders (Wang et al., 2020); however, whether the effect of APO may differ between 2 different reproductive performance properties breeders is still not known.

Therefore, the objective of this study was to verify the hypothesis that dietary APO supplementation could promote the reproductive performance of broiler breeders, and whether this effect will be different between broilers with different egg laying rates.

## MATERIALS AND METHODS

### Birds, Experimental Design

This study was approved by the guidelines of the Animal Care and Use Committee of Sichuan Agricultural University. A total of 256 Arbor Acres broiler breeders (48 wk of age) were selected from Suining commercial farm according to their laying rate. A 2 × 2 factorial design which included 2 egg laying rates (average [AR, 80.45 ± 0.91%] and low [LR, 70.61 ± 1.16%]) and 2 different dietary groups (control [no additive], 200 mg/kg APO [APO]) was used. The APO product was obtained from Hebei Kena Biological Technology Co. Ltd. (Hebei, China); it contained 70% of pectic oligosaccharides and 30% of corn starch. Broiler breeders were fed a complete feeding mixture in mash form (Table 1), and were provided ad libitum access to water and restricted feeding (154 g/day per breeder). The total experimental period was 8 wk. There were 8 replicates with 8 birds per replicate. All birds were housed individually in stainless steel battery cages in a temperature-controlled room on a 16L:8D photo-period.

### Productive Performance and Sample Collection

Egg number, total egg weight, and unqualified eggs (egg weight <50 g or >75 g, misshaped egg, dirty egg, and sand shelled egg) of each replicate were recorded daily. Feed conversion ratio was calculated as the ratio of total feed intake in grams to the total egg weight in grams. Egg production was expressed as an average day production. The qualified egg rate was defined as the ratio of the total number of qualified eggs to the total number of laid eggs per treatment. At the end of 8 wk, 32

**Table 1.** Composition and nutrient level of basal diet (as-fed basis).

Item, %	Amount
Corn	69.50
Soybean meal, 43%	19.00
Soybean oil	1.00
Calcium carbonate	8.25
Calcium hydrophosphate	1.14
L-Lysine hydrochloride	0.08
DL-Methionine	0.11
Threonine	0.02
NaCl	0.30
Choline chloride, 50%	0.10
Vitamin and mineral premix <sup>1</sup>	0.50
Total	100.00
Analyzed nutrient levels, %	
ME, <sup>2</sup> kcal/kg	2,780.00
Crude protein	13.80
Calcium	3.40
Available phosphorus	0.30
Lysine	0.74
Methionine	0.34
Methionine + cysteine	0.59
Threonine	0.54

<sup>1</sup>Provided per kilogram of diet: vitamin A, 12,000 IU; vitamin D<sub>3</sub>, 4,000 IU; vitamin E, 100 mg; vitamin K<sub>3</sub>, 4.0 mg; thiamin, 3.0 mg; riboflavin, 11.5 mg; pyridoxine, 7.2 mg; vitamin B<sub>12</sub>, 0.02 mg; folic acid, 10.8 mg; niacin, 47.1 mg; pantothenic acid, 21.6 mg; biotin, 0.6 mg; iron, 80 mg; copper, 20 mg; manganese, 82.5 mg; zinc, 100 mg; selenium, 0.30 mg; iodine, 1.20 mg.

<sup>2</sup>Calculated according to NRC (1994).

breeders (8 replicates for each treatment) were individually weighted and blood samples were collected from the wing vein in a sterile syringe. Samples were then centrifuged at 3,000 × *g* for 15 min, and serum was stored at −20°C till analysis. After blood collection, broiler breeders were sacrificed by CO<sub>2</sub> suffocation; the ovarian tissues (ovary cortex) and magnum of oviduct were collected and stored at −80°C till gene expression analysis.

### Egg Quality and Egg Hatchability Performance

Egg samples (4 eggs/replicate, 32 eggs/treatment) were collected to analyze the egg quality at the end of the supplementation period (8 wk). Eggshell strength was evaluated by using an eggshell force gauge model II, whereas shell strength was determined by an eggshell thickness gauge (Robotmation Co., Ltd., Tokyo, Japan). Egg internal quality (including Haugh unit [HU], albumen height, and yolk color) was analyzed by an Egg Multi-Tester (EMT-7300, Robotmation Co., Ltd.). Albumen or eggshell ratio was computed as 100 × (albumen weight or eggshell weight [g]/egg weight [g]).

All eggs were collected for 5 consecutive days at the end of the experiment, labeled and weighted individually, and then stored at 15°C until incubation. Eggs were incubated in a commercial hatchery (Jinling JLZ-2, Yaan, China). Fertility was expressed as the ratio of fertile eggs to the total eggs set. The number of eggs that hatched was recorded after 21 d of incubation. Embryonic mortality of eggs was expressed as the ratio of

**Table 2.** Related gene and primer information.

Genes	Orientation	Primer sequences (5'-3')	Product size	Accession number
<i>β-Actin</i>	Forward	GCTACAGCTTCACCACCACA	90	NM_205518.1
	Reverse	TCTCCTGCTCGAAATCCAGT		
<i>GAPDH</i>	Forward	TGGGAAGCTTACTGGAAATGG	88	NM_204305.1
	Reverse	CTTGGCTGGTTTCTCCAGAC		
<i>Caspase 9</i>	Forward	TATGGTGGAGGACATGCAGA	99	XM_424580.5
	Reverse	AATATTGGGAAGGCCTGCTT		
<i>Caspase 3</i>	Forward	AAAGATGGACCACGCTCAGG	204	NM_204725
	Reverse	TGAACGAGATGACAGTCCGG		
<i>Caspase 8</i>	Forward	GCTGTATCCTATCCCACG	125	KM_016991
	Reverse	TCATCAGGCACTCCTTT		
<i>Bax</i>	Forward	GTACGTC AATGTGGTCACCC	210	XM_015274882
	Reverse	TGGGATAATGCTGGGGTTGA		
<i>Bcl-2</i>	Forward	ACCATGAATGAAACCGTGCC	181	NM_205339.2
	Reverse	TTGTCGTAGCCTCTTCTCCC		
<i>PCNA</i>	Forward	GGCGTCAACCTAAACAGCAT	169	NM_204170
	Reverse	GCTCCACATCGAGGTCCATA		

Abbreviations: *Bax*, B lymphoma 2-associated X protein; *Bcl-2*, B lymphoma cell 2; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase; *PCNA*, proliferating cell nuclear antigen.

mortalities to set eggs. Hatchability of set eggs was calculated as the ratio of hatching chicks to set eggs.

### Analysis of Serum Reproductive Hormones

Serum concentrations of estradiol 2, follicle stimulating hormone, testosterone, Anti-Müllerian hormone (AMH), and progesterone were assessed by ELISA test kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following the manufacturer's protocol.

### Serum Inflammatory Cytokine Assay

Serum interleukin (IL)-1 $\beta$ , IL-6, IL-8, IL-10, immunoglobulin A, and immunoglobulin G levels were analyzed with a commercially available ELISA kit (Nanjing Jiancheng Bioengineering Institute) according to the manufacturer's instructions.

### Magnum and Ovary Antioxidant Capacity

The enzymatic activities of superoxide dismutase (SOD), total antioxidant capacity (T-AOC), and malondialdehyde (MDA) content in the ovary and magnum were measured by means of commercial kits (SOD, A001-1; T-AOC, A015; MDA, A003-1; Nanjing Jiancheng Biotechnology Institute, Nanjing, China).

### Apoptosis Assay of Ovary by Terminal Deoxynucleotidyl Transferase dUTP Nick End Labeling Method

Ovaries were quickly removed and placed immediately into methyl aldehyde, and were histochemically stained using terminal deoxynucleotidyl transferase dUTP nick end labeling technique by an in situ apoptosis detection

**Table 3.** Effect of APO on reproduction performance of broiler breeders with different egg laying rates.

Item <sup>1</sup>		Laying rate, %	Egg weight, g	FCR	Qualified egg rate, %
Laying	APO				
AR	-	76.22	65.86	3.17	92.25
AR	+	75.23	67.37	3.02	92.30
LR	-	70.68	66.23	3.32	86.84
LR	+	72.07	68.32	3.20	87.32
SEM		1.28	1.55	0.41	0.07
P-value		0.01	0.19	<0.01	0.56
Main effect					
Laying	AR	75.72	66.62	3.15	91.27
	LR	71.38	67.77	3.28	86.08
APO	-	73.60	66.54	3.26	89.54
	+	74.50	67.84	3.14	89.81
SEM		0.90	1.10	0.29	0.05
P-value					
Laying		<0.01	0.04	0.01	0.03
APO		0.29	0.03	<0.01	0.95
Laying*APO		0.77	0.94	0.61	0.44

Abbreviations: APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; FCR, feed conversion ratio; LR, low egg laying rate.

<sup>1</sup>Each mean represents 8 layers/replicate, 8 replicates/treatment.

**Table 4.** Effect of APO on egg quality of broiler breeders with different egg laying rates.

Item <sup>1</sup>	Eggshell strength, kg/cm <sup>3</sup>	Albumen height, mm	Yolk color	Haugh unit	Yolk weight ratio, %	Eggshell weight ratio, %	Eggshell thickness, mm <sup>-2</sup>	Albumen weight ratio, %
Laying								
AR	3.68	6.01	8.07	74.6	33.67	9.68	30.70	56.65
AR	3.07	7.21	8.13	82.31	33.25	10.03	28.61	56.72
LR	3.45	6.38	8.40	76.49	33.81	10.01	29.52	56.18
LR	3.44	6.97	8.44	79.49	33.05	9.97	30.18	56.98
SEM	0.19	0.30	0.17	1.98	0.36	0.15	0.55	0.36
<i>P</i> -value	0.17	0.03	0.29	0.04	0.40	0.32	0.05	0.46
Main effect								
Laying	3.38	6.61	8.10	78.45	33.46	9.85	29.66	56.69
LR	3.45	6.68	8.42	77.99	33.43	9.99	29.85	56.58
APO	3.56	6.2	8.23	75.55	33.74	9.84	30.11	56.42
+	3.26	7.09	8.28	80.90	33.15	10.00	29.40	56.85
SEM	0.13	0.21	0.12	1.40	0.25	0.11	0.39	0.25
<i>P</i> -value	0.71	0.82	0.06	0.81	0.93	0.37	0.73	0.77
Laying	0.11	<0.01	0.76	0.01	0.10	0.31	0.20	0.23
APO	0.12	0.32	0.95	0.24	0.64	0.19	0.31	0.31
Laying*APO								

Abbreviations: APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; LR, low egg laying rate.

<sup>1</sup>Each mean represents 4 eggs/replicate, 8 replicates/treatment.

kit (Roche, Switzerland). BA200 Digital (Mike Audi Industrial Group Co., Ltd., Guangdong, China) was used for image acquisition. The apoptotic color was light yellow or brown yellow, and negative expression was represented in blue with a white background. Totally, 100 images were considered to measure the cell apoptosis, and the apoptosis rate was defined as the percentage of apoptotic cells in 100 cells counted.

### Ovary Apoptosis-Related mRNA Expression by Real-Time PCR

Total RNA was extracted with TRIzol reagent (TaKaRa, Dalian, China) according to the manufacturer's instructions. cDNA was synthesized via reverse transcription, which was performed with 2 µg of total RNA using a PrimeScript RT Reagent Kit with gDNA Eraser (TaKaRa). Quantitative real-time PCR was performed on an ABI Prism 7000 detection system in a 2-step protocol with SYBR Green (TaKaRa). Each 10 µL volume reaction contained 1 µL of cDNA, 5 µL of SYBR Premix Ex Taq (2×), 0.2 µL of ROX reference dye (50×), 0.4 µL of each forward and reverse primer, and 3 µL of PCR-grade water. The thermal cycling program included a 1-min preincubation at 95°C, followed by 40 cycles of denaturation at 95°C for 5 s, a 60°C annealing step for 25 s, and an extension at 72°C for 15 s. Gene expression of *caspase 3*, *caspase 8*, *caspase 9*, *Bcl-2*, *Bax*, and *PCNA* was determined by quantitative real-time PCR in the ovary of broiler breeders. The primer information of all the genes is listed in Table 2. Each sample was assayed in triplicate and 2 housekeeping genes (β-actin and GAPDH) were assessed for the stability of expression. Gene expression was calculated by using the 2<sup>-ΔΔCT</sup> method.

### Statistical Analysis

Data were analyzed as a 2 × 2 factorial using the general linear model procedures of SAS 9.2 (SAS Institute, Cary, NC), with a model that included the main effects of egg laying rate and APO, as well as their interaction. Means were compared by using Tukey's range test to determine significant differences among means with a significant level of *P* < 0.05.

## RESULTS

### Reproduction Performance, Egg Quality, and Incubation Performance

The LR breeders presented higher egg weight but lower egg laying rate, qualified egg rate, and feed efficiency than the AR breeders (*P*<sub>(laying)</sub> < 0.05; Table 3). No difference was found in egg quality between LR and AR breeders (*P*<sub>(laying)</sub> > 0.05; Table 4). Dietary supplementation with APO improved egg weight, feed efficiency, and resulted in higher albumen height and HU in both breeders compared with no APO addition (*P*<sub>(APO)</sub> < 0.05).

**Table 5.** Effect of APO on hatchability of broiler breeders with different egg laying rates.

Item <sup>1</sup>		Fertility, %	Hatchability of set eggs, %	Health chicken rate, %	Embryonic mortality, %
Laying	APO				
AR	–	96.29	87.69	87.58	8.49
AR	+	96.25	86.88	86.25	4.38
LR	–	97.10	89.52	89.66	7.55
LR	+	94.48	88.51	88.51	7.04
SEM		1.64	2.83	2.67	2.41
<i>P</i> -value		0.7	0.90	0.81	0.95
Main effect					
Laying	AR	96.24	87.30	86.89	6.97
	LR	95.79	89.05	89.06	7.31
APO	–	96.76	88.69	88.69	8.07
	+	95.37	87.69	87.38	5.71
SEM		1.16	2.01	1.90	2.71
<i>P</i> -value					
Laying		0.79	0.55	0.46	0.64
APO		0.41	0.73	0.63	0.8
Laying*APO		0.43	0.97	0.94	0.94

Abbreviations: APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; LR, low egg laying rate.

<sup>1</sup>Each mean represents 8 layers/replicate, 8 replicates/treatment.

There were no significant differences in embryo mortality, fertility, hatchability of set eggs, and healthy born chicken rate between the AR and LR breeders; moreover, no effect of APO supplementation was noted on the incubation performance parameters measured in this study (Table 5,  $P > 0.05$ ).

### Serum Hormone, Cytokine, and Immunoglobulins Concentration

No differences in the serum concentration of the measured hormones (estradiol 2, follicle stimulating hormone, testosterone, and progesterone), cytokines (IL-1 $\beta$ , IL-6, IL-8, IL-10), and immunoglobulin A, immunoglobulin G were detected between the AR and LR breeders ( $P_{(\text{laying})} > 0.05$ ; Table 6, Table 7); however, the LR breeders demonstrated decreased

serum AMH and leptin levels than the AR breeders ( $P_{(\text{laying})} \leq 0.05$ ). Dietary supplementation with APO significantly decreased the pro-inflammatory cytokine levels (IL-1 $\beta$ , IL-8) in the 2 egg laying rate breeders ( $P_{(\text{APO})} < 0.05$ ).

### Antioxidant Capacity of Magnum and Ovary

The activities of antioxidant enzymes (SOD, T-AOC) in the ovary were lower, while MDA was higher in LR breeders than in the AR breeders ( $P_{(\text{laying})} \leq 0.05$ ; Table 8). Dietary supplementation with APO significantly increased the SOD and T-AOC activities, and decreased the MDA contents of the ovary in the 2 egg laying rate breeders ( $P_{(\text{APO})} < 0.05$ ). The APO were also observed to increase SOD activity in magnum ( $P_{(\text{APO})} < 0.05$ ), while no difference was observed in

**Table 6.** Effect of APO on blood hormone levels of broiler breeders with different egg laying rates.

Item <sup>1</sup>		E2, pmol/L	FSH, U/L	Testosterone, nmol/L	AMH, pg/mL	Leptin, ng/L	Progesterone, pmol/L
Laying	APO						
AR	–	69.33	8.04	209.34	286.45 <sup>a</sup>	411.08 <sup>b</sup>	1,829.47
AR	+	68.52	7.95	187.27	270.52 <sup>a</sup>	358.30 <sup>b</sup>	1,708.83
LR	–	73.37	7.51	190.45	168.78 <sup>b</sup>	566.22 <sup>a</sup>	1,820.09
LR	+	72.15	7.54	186.03	241.99 <sup>a</sup>	419.05 <sup>b</sup>	1,875.3
SEM		4.07	0.38	12.15	17.75	20.75	130.86
<i>P</i> -value		0.26	0.09	0.29	<0.01	0.02	0.83
Main effect							
Laying	AR	69.46	7.99	197.87	278.48	383.09	1,769.15
	LR	72.74	7.52	189.31	201.38	403.09	1,847.69
APO	–	72.89	7.83	190.03	277.61	397.51	1,824.78
	+	71.42	7.7	181.15	271.25	388.68	1,792.07
SEM		2.88	0.27	8.59	14.48	19.55	92.53
<i>P</i> -value							
Laying		0.13	0.28	0.25	0.05	0.04	0.56
APO		0.71	0.27	0.14	0.72	0.75	0.81
Laying*APO		0.2	0.14	0.73	0.52	0.32	0.51

Abbreviations: AMH, Anti-Müllerian hormone; APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; E2, estrogen 2; FSH, follicle stimulating hormone; LR, low egg laying rate.

<sup>1</sup>Each mean represents 2 layers/replicate, 8 replicates/treatment.



**Table 7.** Effect of APO on blood cytokine levels of broiler breeders with different egg laying rates.

Item <sup>1</sup>		IL-1 $\beta$	IL-6	IL-8	IL-10	IgA	IgG
Laying	APO						
AR	-	30.31	5.97	33.04	8.53	67.30	11.72
AR	+	24.32	6.08	26.35	8.85	64.41	34.56
LR	-	30.56	6.46	29.49	8.78	68.61	22.81
LR	+	27.61	6.09	25.78	8.31	53.54	40.23
SEM		2.07	0.60	3.10	0.87	6.00	13.57
<i>P</i> -value		0.09	0.92	0.29	0.95	0.179	0.42
Main effect							
Laying	AR	27.32	6.03	29.70	8.69	65.86	23.14
	LR	29.10	6.27	27.64	8.54	61.08	31.52
APO	-	30.45	6.21	31.27	8.65	67.96	17.26
	+	25.96	6.08	26.07	8.58	58.97	37.39
SEM		1.39	0.40	2.08	0.58	3.94	8.76
<i>P</i> -value							
Laying		0.35	0.65	0.46	0.85	0.40	0.62
APO		0.03	0.81	0.05	0.93	0.28	0.12
Laying*APO		0.42	0.66	0.59	0.61	0.26	0.83

Abbreviations: APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; IgA, immunoglobulin A; IgG, immunoglobulin G; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; LR, low egg laying rate.

<sup>1</sup>Each mean represents 2 layers/replicate, 8 replicates/treatment.

magnum T-AOC and MDA between AR and LR breeders ( $P_{\text{laying}} > 0.05$ ).

### Ovary Apoptosis Rate and mRNA Expression of Ovary Apoptosis-Related Genes

The apoptosis rate of ovary was higher in LR breeders than in the AR breeders ( $P_{\text{laying}} < 0.01$ ; Figure 1),

while dietary APO treatment had no effect on the apoptosis in broiler breeders ( $P > 0.01$ ). The pro-apoptosis-related gene expression (*caspase 9* and *Bax*) in the ovary of LR breeders was higher, while anti-apoptosis-related gene expression (*Bcl-2*, *PCNA*) was lower in LR compared with the AR breeders ( $P_{\text{laying}} < 0.05$ ; Figure 2). Dietary supplementation with APO decreased the *caspase 9* and *Bax* expression in LR breeders ( $P_{\text{interaction}} = 0.05$ ), and increased the *Bcl-2* and *PCNA* expression in both 2the breeders ( $P_{\text{APO}} < 0.05$ ).

## DISCUSSION

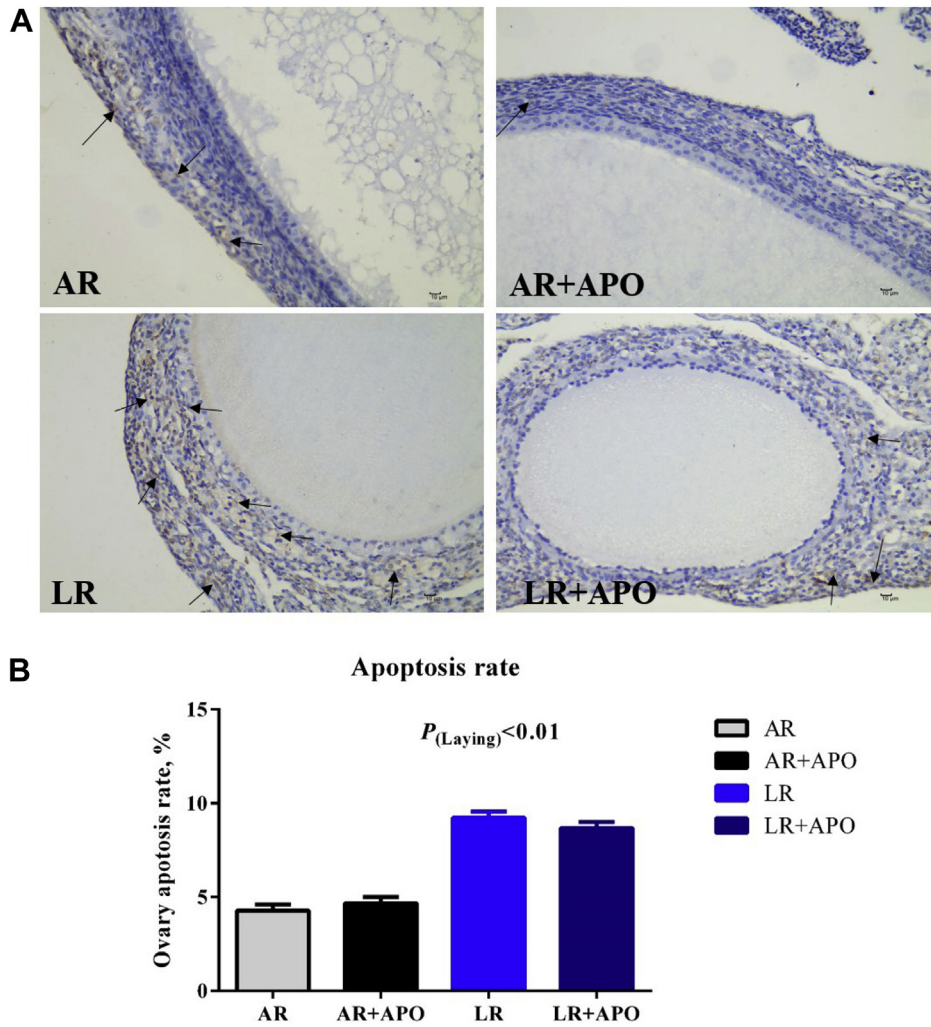
Generally, there are several factors that could affect animal health and reproduction performance such as genetics, nutrition, age, and the rearing environment of animals (Bova et al., 2014; Shi et al., 2020). The reason why the reproduction performance differs even when breeders share the same genetic background, diet, and house environment is not clear. In our study, the production performance (egg laying rate and feed efficiency) of AR breeders was higher than that of LR breeders. Intestine health and microbiota balance are important for nutrient digestion, absorption, and utilization in poultry. Torok et al. (2011) also showed that the intestinal microbiota structure was significantly different between broilers with feed efficiency and this may cause differences in nutrient digestion and utilization. As a functional oligosaccharide, pectic oligosaccharides have been found to regulate the physiological function of humans and animals, such as antioxidant capacity, immune function, lipid metabolism, and intestinal microflora (Li et al., 2010, 2013; Gullnón et al., 2013). In the

**Table 8.** Effect of APO on magnum and ovary antioxidant capacity of broiler breeders with different egg laying rates.

Item <sup>1</sup>		Magnum			Ovary		
		SOD	T-AOC	MDA	SOD	T-AOC	MDA
Laying	APO						
AR	-	33.28	0.16	0.54	189.21	0.66	1.33
AR	+	39.46	0.20	0.57	245.11	0.76	0.78
LR	-	31.10	0.16	0.48	122.31	0.54	2.54
LR	+	35.58	0.15	0.35	167.40	0.67	1.88
SEM		2.32	0.02	0.08	25.6	0.10	0.20
<i>P</i> -value		0.10	0.50	0.22	0.01	0.03	<0.01
Main effect							
Laying	AR	36.37	0.18	0.55	216.09	0.72	1.56
	LR	33.34	0.16	0.41	154.76	0.56	2.63
APO	-	32.19	0.16	0.51	155.71	0.56	1.98
	+	37.52	0.17	0.46	209.54	0.76	1.33
SEM		1.64	0.02	0.06	19.54	0.09	0.12
<i>P</i> -value							
Laying		0.21	0.37	0.09	0.01	0.04	0.05
APO		0.03	0.56	0.5	0.03	0.03	<0.01
Laying*APO		0.72	0.28	0.35	0.72	0.28	0.35

Abbreviations: APO, 200 mg/kg apple pectic oligosaccharide; AR, average egg laying rate; LR, low egg laying rate; MDA, malondialdehyde; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

<sup>1</sup>Each mean represents 2 layers/replicate, 8 replicates/treatment.

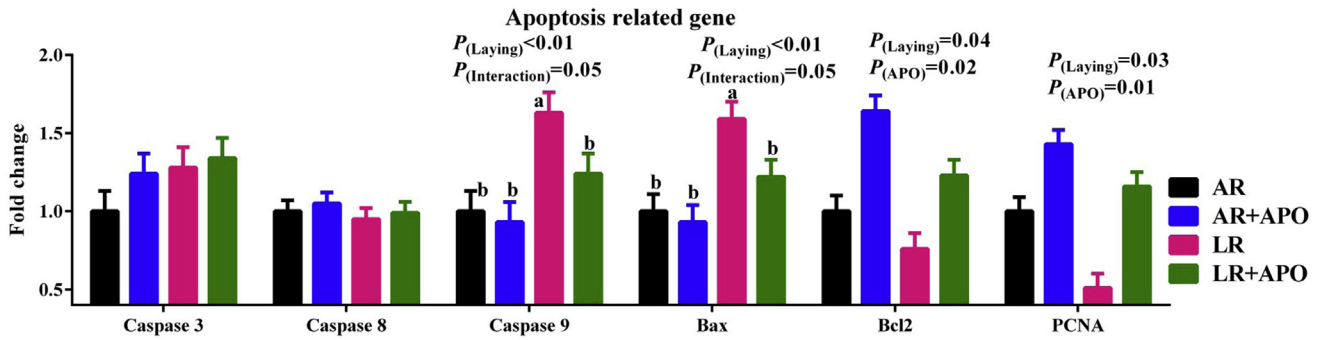


**Figure 1.** The effect of APO on ovary apoptosis of broiler breeders with different egg laying rates. Each means represents 2 layers/replicate, 8 replicates/treatment. (A) Ovary apoptosis (TUNEL). Apoptotic color is light yellow or brown yellow (as shown by black arrow), negative expression of blue, white background. (B) Ovary apoptosis rate. Abbreviations: APO, apple pectic oligosaccharide; AR, average egg laying rate; LR, low egg laying rate; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling.

current study, we found that dietary supplementation with APO increased reproductive performance (egg weight and feed efficiency) and albumen quality (albumen height and HU), but did not influence the egg laying rate. The positive effect of oligosaccharide on egg weight and feed efficiency could be attributed to enhanced breeders' health and improved gastrointestinal functionality (Celi et al., 2019; Mao et al., 2021). Literature about the effect of APO on broiler breeders is limited. In our previous study, we also found that APO can improve egg weight and albumen quality in breeders (Zhao et al., 2019). It has been shown that mannan-oligosaccharides could improve intestine health to enhance nutrient retention, and therefore improve the feed efficiency in laying hens (Gunal et al., 2006; Jahanian and Ashnagar, 2015). Also, APO was found to improve gut health and the reproduction performance of rats (Liu et al., 2020), whereas it does alleviate growth performance, diarrhea, and gut barrier function of finishing pigs under rotavirus infection (Mao et al., 2016, 2017; Chen et al., 2017). Since egg albumen was mainly

secreted in the magnum of the laying hen's oviduct, the structural and functional integrity of magnum plays a determinant role in its quality. We also found that APO increased the T-AOC and T-SOD activity, and reduced the lipid peroxidation product (MDA) concentration in the magnum and ovary. Thus, APO, promoting the antioxidant capacity of magnum, could be one of the reasons for APO to improve the albumen quality in the current study. Similarly, previous studies showed that APO administration can promote the antioxidant capacity in serum and intestine of rats and weaning pigs (Mao et al., 2016, 2019; Chen et al., 2017).

Pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-12, IFN- $\gamma$ , IL-18, and tumor necrosis factor- $\alpha$ ) play a central role in inflammatory diseases of infectious or non-infectious origin, and they also cause widespread effects on metabolism, involving alterations in lipid, carbohydrate, and protein metabolism (Grimble, 2013). Although a pro-inflammatory response is part of natural immune defense, it is important to note that an unabated pro-inflammatory cascade for a longer duration



**Figure 2.** The effect of APO on ovary apoptosis-related gene expression of broiler breeders with different egg laying rates. Each means represents 2 layers/replicate, 8 replicates/treatment; a, b means with different superscript within a bar differ significantly ( $P < 0.05$ ). Abbreviations: APO, apple pectic oligosaccharide; AR, average egg laying rate; *Bax*, B lymphoma 2 associated X protein; *Bcl-2*, B lymphoma cell 2; LR, low egg laying rate; *PCNA*, proliferating cell nuclear antigen.

may lead to cellular injury by excessive generation of reactive oxygen species (Blaser et al., 2016). In the current study, we found that APO decreased the pro-inflammatory cytokine levels (IL-1 $\beta$ , IL-8) in serum. Our previous study also showed that APO administration may regulate Th cytokine generation, and attenuate the rotavirus-induced inflammation in weaned piglets (Chen et al., 2017). These results declare that APO decreased the pro-inflammatory cytokine to reduce the inflammatory response and immune overreaction, so that nutrients can be more favorably distributed for growth and reproduction.

Ovarian follicle selection and atresia are the main determinant factors associated with egg laying, and this process is strictly controlled by neuroendocrine regulation (Johnson, 2012). Serum AMH is expressed by granulosa cells of developing follicles and is a biomarker for ovarian follicle reserve and reproductive performance (Pangas, 2012). In this study, LR breeders had lower serum AMH and higher leptin levels, which indicate that LR breeders had lower ovarian reserve. In our previous study, interestingly, we also found that low reproductive breeders exhibited higher abdominal fat rates (Zhao et al., 2019), which may indicate that fat deposition may affect fertility. Leptin, which is secreted by adipose tissue in overweight animals, is also at a higher level in our study. It has also been reported that leptin can directly antagonize ovarian estradiol and progesterone secretions (Agarwal et al., 1999; Lei et al., 2014). Therefore, this also may be the reason why the follicle development of the LR group breeders is not as good as that of AR group breeders in our study.

Follicle atresia in the late laying phase (35–65 wk of age) may be the main contributing factor for the inferior total laying performance and the early culling in practice. Apoptosis in follicle granulosa cells may be one of the main reasons that led to follicle atresia and ovary atrophy (Hussein, 2005; Regan et al., 2016). In the current study, we observed that the ovary apoptosis rate was higher in LR breeders. Pro-apoptotic caspases (*caspase 2, 3, 6, 7, 8, 9, 10*) and *Bax* are known to be

mainly involved in mediating cell death signaling transduction, whereas *Bcl-2* and *PCNA* play a key role in reducing cell apoptosis (Li and Yuan, 2008; Melo et al., 2015). In the present study, we found that pro-apoptosis-related gene expression (*caspase 9* and *Bax*) was also upregulated in low reproductive performance breeders. A study in pigs has reported an intensive expression of *caspase 9* mRNA in the granulosa cells of early atretic and progressed atretic follicles but not in the granulosa cells of healthy follicles (Matsui, 2003). In our study, the relative expression of pro-apoptosis factors in the ovary of AR breeders was lower than that of LR breeders, suggesting that the number of atretic follicles in the AR breeders might have been lower than that of LR breeders; while this hypothesis needs to be confirmed in future studies, it could provide an explanation for the different egg laying rates between the 2 groups of breeders. At the same time, APO was found to decrease *caspase 9* and *Bax* expression in LR breeders and increase the *Bcl-2* and *PCNA* expression in the 2 breeders, which may be associated with the low pro-inflammatory cytokine levels and higher antioxidant capacity that was caused by APO administration. These results may indicate that APO can improve ovary function in both breeders. Further study is necessary to explore its mechanism in alleviating the follicle atresia of birds.

## CONCLUSION

Our results suggest that breeders that had a lower egg laying rate exhibit lower antioxidant capacity and ovary function, while dietary supplementation with APO might improve albumen quality and antioxidant capacity, and decrease the inflammatory factors expression and ovary apoptosis-related genes to improve ovary function in lower reproductive breeders.

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## DISCLOSURES

No conflict of interest exists in the submission of this manuscript, and the manuscript is approved by all authors for publication.

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