The effect of dietary pectic oligosaccharide supplementation on intestinal health of broiler breeders with different egg-laying rates

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ABSTRACT This study was conducted to explore whether dietary pectic oligosaccharide (**POS**) supplementation could improve gut health of broiler breeders with different egg-laying rates. A 2 × 2 factorial design was used in this study. Two hundred fifty-six Arbor Acres broiler breeders (48 wk of age), including 128 average egg-laying rate and 128 low egg-laying rate (**LELR**) birds, were randomly fed with the diets supplemented with or without 200 mg kg^{-1} of POS (n = 8). The trial lasted for 8 wk. Compared with average egg-laying rate broiler breeders, LELR broiler breeders had lower laying rate and qualified egg rate ($P < 0.05$), higher egg weight and feed conversion ratio ($P < 0.05$), higher malondialdehyde (**MDA**) levels in the jejunum ($P < 0.05$), higher *IL-6* ($P < 0.05$) and tumor necrosis factor α (***TNF-α***) ($P = 0.07$) mRNA expressions in the jejunal mucosa, and lower microflora diversity in cecal digesta. Dietary POS supplementation increased egg weight of broiler breeders ($P < 0.05$), enhanced superoxide dismutase activity in the jejunum ($P < 0.05$), decreased MDA level in the jejunum ($P < 0.05$), upregulated zonula occluden 1 mRNA expression in the jejunal mucosa ($P < 0.05$), downregulated *IL-6* and *TNF-α* mRNA expressions in the jejunal mucosa ($P < 0.05$), and regulated relative abundance of some microbiota (including the phylum and genus, $P < 0.05$). In addition, in LELR broiler breeders, POS administration enhanced villus height ($P = 0.08$) and *ZO-2* mRNA expression ($P = 0.09$) in the jejunal mucosa, alleviated the increasing MDA level in the jejunum ($P < 0.05$) and *IL-6* and *TNF-α* mRNA expressions in the jejunal mucosa ($P < 0.05$), and regulated relative abundance of some microbiota (including the phylum and genus, $P < 0.05$). These results suggest that supplementing POS in diets may elevate gut health via improvement of intestinal barrier function, antioxidant capacity, and microbiota composition in broiler breeders with different egg-laying rates.

Key words: pectic oligosaccharide, broiler breeder, different egg-laying rate, gut health

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INTRODUCTION

As a functional oligosaccharide, pectic oligosaccharide (**POS**) may regulate some physiological functions of animals, which results in its potentials as antibiotic substitutes. Its main components are pectic disaccharide and trisaccharide that contain galacturonic acid. The previous studies reported that POS administration could regulate lipid metabolism and antioxidant capacity in hyperlipidemic mice induced by high-fat diet (Li et al., 2010), improve meat quality of finishing pigs (Mao et al., 2017a), and affect intestinal microbiota of humans and pigs in in vitro experiments (Leijdekkers et al., 2014). Importantly, recent studies in our laboratory have shown that dietary POS supplementation is beneficial for nonspecial gut barrier function, antioxidant capacity, immunity, and microflora in weaned rats and piglets (Mao et al., 2016; Chen et al., 2017), and relieves the negative effect of rotavirus infection on gut health, diarrhea, and growth performance in piglets (Mao et al., 2017b, 2019a).

Reproductive performance is a key point to broiler breeders (Rozenboim et al., 2007), which makes the relative research focus on reproductive organs (such as the ovary). Gut health is the important protection mechanism of animal health and production (Mao et al.,

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2011). Reproductive performance could be associated with gut health. Under the same conditions (including genetics, nutrition, age, management, and environment), broiler breeders always have different reproduction performances (Rozenboim et al., 2007; Shi et al., 2020). Thus, it is possible that utilization and reproduction of broiler breeders were improved via the increase in gut health.

Our previous studies also indicated that POS administration could elevate reproductive performance in original female rats (Liu et al., 2020) and improve albumen height and Haugh units in breeders (Zhao et al., 2019). The aim of this study was to analyze the hypothesis that dietary POS supplementation can, to some extent, improve reproductive performance and gut health in broiler breeders (especially broiler breeders with low egg-laying rate [**LELR**]).

MATERIALS AND METHODS

Animals and Diets

All broiler breeder procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of Sichuan Agricultural University and approved by the Animal Ethics Committee of Sichuan Agricultural University (Chengdu, China).

Two hundred fifty-six Arbor Acres broiler breeders (48 wk of age) from Suining commercial farm were used in this experiment. Half of these birds were average egg-laying rate (**AELR**; approximately 80%) ones, whereas half were LELR ones (about 71%). All broiler breeders had restricted feeding (154 g d^{-1} for every breeder) and free access to water.

The diets were formulated to meet the NRC (1994) nutrient requirements. The basal diet is presented in Table 1. The POS-supplemented diet was the basal diet with 200 mg kg^{-1} of POS product, which was added by replacing the same amount of corn. This POS product is derived from apple pectin and purchased from Hebei Kena Biological Technology Co. Ltd. (Hebei, China), in which POS and corn starch contents were 30 and 70%, respectively.

Table 1. The composition and nutrient content of basal diets.

Ingredients	Content, %
Corn	69.50
Soybean meal	19.00
Soybean oil	1.00
$CaCO_3$	8.25
$CaHPO_4$	1.14
L-Lysine·HCl	0.08
DL-Methionine	0.11
L-Threonine	0.02
NaCl	0.30
Choline chloride, 50%	0.10
Vitamin and mineral premix[1]	0.50
Total	100.00
Nutrient levels,[2] %	
Metabolic energy, kcal/kg	2,780.00
CP	13.80
Calcium	3.40
Available phosphorus	0.30
Lysine	0.74
Methionine	0.34
Methionine + cysteine	0.59
Threonine	0.54

[1]Provided the following per kilogram of diet: vitamin A, 12,000 IU; vitamin D_3, 4,000 IU; vitamin E, 100 mg; vitamin K_3, 4.0 mg; thiamin, 3.0 mg; riboflavin, 11.5 mg; pyridoxine, 7.2 mg; vitamin B_{12}, 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.
[2]Nutrient levels represent the calculated values.

Experimental Design and Sample Collection

A 2 × 2 factorial design, including 2 egg-laying rate levels and 2 different diet treatments, was used in this trial. After 3 d of acclimation, half of the broiler breeders with AELR and LELR were randomly fed with the POS-supplemented diet, and the other broiler breeders with AELR and LELR were fed with the basal diet (n = 8, 8 breeders per replicate). The whole duration was 8 wk. All birds were individually housed at 22°C and subjected to a 16L:8D photoperiod.

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

On day 57, thirty-two broiler breeders (8 replicates per treatment) were slaughtered by CO_2 suffocation. The intestine was removed. The jejunum was quickly separated and flushed with sterile ice-cold saline. Jejunal segments (about 2 cm) were fixed in 4% paraformaldehyde for mucosal morphology, and another 2-cm jejunum segments were collected for measuring antioxidant capacity. The jejunal mucosa was also gathered by scraping the gut wall using a sterile glass microscope slide. The cecal digesta (about 3 g) were collected in sterile tubes. These samples of jejunum segments, jejunal mucosa, and cecal digesta were immediately frozen in liquid nitrogen and stored at −80°C until analysis.

Morphology of the Jejunal Mucosa

Morphology of the jejunal mucosa was analyzed as described previously by Mao et al. (2019b). In brief, after fixing in 4% paraformaldehyde, the jejunal segment was embedded in paraffin and stained with hematoxylin and eosin. Villus height and crypt depth were measured at a magnification of 40× using the Olympus CK 40 microscope (Olympus, Tokyo, Japan). A total of 10 intact villi and crypts were randomly selected in each sample. Then, the ratio of villus height to crypt depth was calculated.

Table 2. Primer sequences used for real-time PCR.

Gene	Primer	Nucleotide sequences, 5′–3′
ZO-1	Forward	GAGCGCAAGTTTGAAAGTCC
	Reverse	AGGAGGCTGTGATGAGCTGT
ZO-2	Forward	GAAAGCTCCAGCTGGTTGTC
	Reverse	GGGGAGAACGATCTGTTTGA
mucin 2	Forward	TGCCAGCCTTTTTATGCTCT
	Reverse	AGTGGCCATGGTTTCTTGTC
IL-1β	Forward	GCATCAAGGGCTACAAGCTC
	Reverse	CAGGCGGTAGAAGATGAAGC
IL-6	Forward	CTCCTCGCCAATCTGAAGTC
	Reverse	CCCTCACGGTCTTCTCCATA
IL-8	Forward	GATTGAACTCCGATGCCAGT
	Reverse	TCCACATTCTTGCAGTGAGG
TNF-α	Forward	GCCCTTCCTGTAACCAGATG
	Reverse	ACACGACAGCCAAGTCAACG
IFN-γ	Forward	CAGATGTAGCTGACGGTGGA
	Reverse	CATCGAAACAATCTGGCTCA
β-actin	Forward	GCTACAGCTTCACCACCACA
	Reverse	TCTCCTGCTCGAAATCCAGT

Abbreviations: IFN, interferon; TNF-α, tumor necrosis factor α; ZO-1, zonula occluden 1.

Antioxidant Capacity in the Jejunum

Superoxide dismutase (**SOD**) activity, total antioxidant capacity, and malondialdehyde (**MDA**) levels in the jejunum were analyzed by using commercial kits obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China) according to the manufacturer's instructions.

mRNA Expression Levels of Some Gut Barrier–Related Genes and Cytokines in the Jejunal Mucosa

Total RNA of the jejunal mucosa was extracted using the TRIzol reagent (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) on the basis of the manufacturer's instructions. RNA concentration was determined by using DU 640 UV spectrophotometer detection (Beckman Coulter Inc., Fullerton, CA), and the ratio of OD_{260} to OD_{280} was found to be 1.8–2.0. RNA quality was assessed by 1% agarose gel electrophoresis. The cDNA of samples was synthesized by using the PrimeScript RT reagent kit and gDNA Eraser (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China). The primers of genes, listed in Table 2, were purchased from TaKaRa Biotechnology (Dalian) Co., Ltd. (Dalian, China). The mRNA expressions of genes in all samples were analyzed by real-time quantitative PCR using SYBR Premix Ex Taq reagents (TaKaRa Biotechnology (Dalian) Co., Ltd., Dalian, China) and a CFX-96 Real-Time PCR detection System (Bio-Rad Laboratories, Richmond, CA) as described previously by Mao et al. (2018). The housekeeping gene (β-actin) was chosen to correct for variance in the amount of RNA input in the reaction. The relative mRNA expression compared with the housekeeping gene was obtained using previous methods (Mao et al., 2018).

Microbiota Analysis in Cecal Digesta

The microbiota in cecal digesta was determined as described previously by Wang et al. (2019). In brief, bacterial DNA was extracted from cecal digesta using the QIAamp DNA Stool Mini Kit (QIAGEN, CA, Hamburg, Germany) according to the manufacturer's instructions. Total DNA was eluted in 50 μL of elution buffer, confirmed by 1.2% agarose gel electrophoresis, and stored at −80°C until analysis via PCR by LC-Bio Technology (Hangzhou, China). Before sequencing, the 16S rDNA V3–V4 region of each sample was amplified with a set of primers targeting the 16S rRNA gene region. Sequencing libraries were generated using the New England Biolabs Next Ultra DNA Library Prep Kit for Illumina (New England Biolabs, MA) following the manufacturer's recommendations, and index codes were added. The library quality was assessed using the Qubit@ 2.0 Fluorometer (Life Technologies, CA) and Agilent Bioanalyzer 2100 system. This library was quantified by Qubit and Q-PCR. After qualification, the library was sequenced using HiSeq2500 PE250. Sequencing and bioinformatics analysis were performed by Novogene Bioinformatics Technology Co. (Tianjin, China).

Statistical Analysis

These data were analyzed as a 2 × 2 factorial with the general liner model procedures of SAS (version 8.1; SAS Institute, Gary, NC). The model factors contained the effects of egg-laying rate (average and low) and POS treatment (with or without POS in diets), as well as their interaction. And means were also compared by using Tukey's range test to determine significant differences. $P < 0.05$ was determined statistically significant, while $P < 0.10$ was regarded as statistical tendency.

RESULTS

Reproductive Performance

The effects of dietary POS supplementation on reproductive performance (including laying rate, egg weight, feed conversion ratio, and qualified egg rate) of broiler breeders with AELR and LELR are shown in Table 3. Compared with the broiler breeders with AELR, the broiler breeders with LELR had lower laying rate and qualified egg rate ($P < 0.05$) and higher egg weight and feed conversion ratio ($P < 0.05$) (Table 3). However, dietary POS supplementation increased egg weight of broiler breeders ($P < 0.05$) and did not affect laying rate, feed conversion ratio, and qualified egg rate ($P > 0.05$) (Table 3). There were no significant interactions to production performance between egg-laying rate and POS administration ($P > 0.05$) (Table 3).

Table 3. The effect of dietary pectic oligosaccharide supplementation on production performance in broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
Laying rate, %	77.82[a]	76.27[a]	70.77[b]	69.51[b]	0.85	<0.05	0.15	0.88
Egg weight, g	65.33[b]	66.64[a,b]	66.80[a,b]	68.09[a]	0.31	<0.05	<0.05	0.99
Feed conversion ratio	3.12[b]	3.07[b]	3.28[a]	3.31[a]	0.03	<0.05	0.86	0.53
Qualified egg rate, %	95.44[a]	93.17[a,b]	90.59[b]	88.86[b]	0.89	<0.05	0.21	0.86

[a,b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet.

Jejunal Mucosa Morphology

As shown in Table 4, there was no significant difference in jejunal mucosa morphology between AELR and LELR broiler breeders ($P > 0.05$). And dietary POS supplementation did not significantly affect jejunal mucosa morphology of broiler breeders ($P > 0.05$, Table 4). Pectic oligosaccharide administration tended to improve villus height in the jejunal mucosa of LELR broiler breeders ($P = 0.08$, Table 4).

Antioxidant Capacity in the Jejunum

The MDA level in the jejunum of broiler breeders with LELR was higher than that in the jejunum of broiler breeders with AELR ($P < 0.05$, Table 5). Supplementing POS in diets enhanced the activity of SOD and decreased the concentration of MDA in the jejunum of broiler breeders ($P < 0.05$, Table 5). However, POS administration alleviated the increasing MDA level in the jejunum of LELR broiler breeders ($P < 0.05$, Table 5).

The mRNA Expression Levels of Some Gut Barrier–Related Genes and Cytokines in the Jejunal Mucosa

The effects of dietary POS supplementation on mRNA expressions of some gut barrier–related genes and cytokines in the jejunal mucosa of broiler breeders with AELR and LELR are shown in Tables 6 and 7. In the jejunal mucosa of broiler breeders with LELR, the mRNA expressions of *IL-6* ($P < 0.05$) and tumor necrosis factor α (***TNF-α***) ($P = 0.07$) were elevated. Pectic oligosaccharide administration increased the mRNA expression of zonula occluden 1 (***ZO-1***) and decreased the mRNA expressions of *IL-6* and *TNF-α* in the jejunal mucosa of broiler breeders ($P < 0.05$). In addition, dietary POS supplementation relieved the increasing mRNA expressions of *IL-6* and *TNF-α* ($P < 0.05$) and tended to improve *ZO-2* mRNA expression ($P = 0.09$) in the jejunal mucosa of LELR broiler breeders.

Alpha Diversity of Microbiota in the Cecal Digesta

As shown in Table 8, the observed species, community richness (Chao1 and ACE), and community diversity (Shannon) indices of the microbiota in the cecal digesta of broiler breeders with LELR were lower than those in the cecal digesta of broiler breeders with AELR ($P < 0.05$). Supplementing POS in diets did not significantly affect alpha diversity of microbiota in the cecal digesta of broiler breeders ($P > 0.05$). And there were no significant interactions to alpha diversity of microbiota in the cecal digesta between egg-laying rate and POS administration ($P > 0.05$).

Beta Diversity of Microbiota in the Cecal Digesta

The effects of dietary POS supplementation on beta diversity of microbiota in the cecal digesta of broiler breeders with AELR and LELR are shown in Figure 1. The microbiota in the cecal digesta of broiler breeders with AELR and LELR was clearly differentiated, but the diversity derived from POS treatment could be hardly observed.

Table 4. The effect of dietary pectic oligosaccharide supplementation on jejunal mucosa morphology in broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
Villus height, μm	1,434.08	1,221.51	1,236.30	1,498.48	62.87	0.75	0.84	0.08
Crypt depth, μm	213.48	189.74	210.73	220.43	10.07	0.55	0.76	0.47
Villus height-to-crypt depth ratio	6.80	6.56	5.89	6.84	0.27	0.59	0.55	0.32

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet.

Table 5. The effect of dietary pectic oligosaccharide supplementation on antioxidant capacity in the jejunum of broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
SOD, U/mg protein	162.20[b]	197.31[a,b]	165.16[a,b]	211.61[a]	8.40	0.57	<0.05	0.71
T-AOC, U/mg protein	1.27	1.28	1.30	1.44	0.05	0.39	0.51	0.55
MDA, nmol/mg protein	0.34[b]	0.31[b]	0.59[a]	0.31[b]	0.03	<0.05	<0.05	<0.05

[a,b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; MDA, malondialdehyde; POS, pectic oligosaccharide–supplemented diet; SOD, superoxide dismutase; T-AOC, total antioxidant capacity.

Microbiota Composition in the Cecal Digesta

We analyzed the relative microbial (phylum and genus) abundances in the cecal digesta of broiler breeders with AELR and LELR. The results are shown in Tables 9 and 10. Compared with the cecal digesta of AELR broiler breeders, the cecal digesta of LELR broiler breeders had higher relative abundances of Bacteroidetes (phylum, $P < 0.05$), Firmicutes (phylum, $P < 0.05$), Euryarchaeota (phylum, $P < 0.05$), and *Methanobrevibacter* (genus, $P < 0.05$) and the lower relative abundances of Proteobacteria (phylum, $P < 0.05$), Spirochaetes (phylum, $P = 0.09$), Actinobacteria (phylum, $P < 0.05$), and *Faecalibacterium* (genus, $P = 0.05$). Dietary POS supplementation increased the relative abundance of *Phascolarctobacterium* (genus, $P < 0.05$) and decreased the relative abundance of Kiritimatiellaeota (phylum, $P < 0.05$) in the cecal digesta of broiler breeders. Moreover, there were significant interactions to the relative abundance of Bacteroidetes (phylum, $P < 0.05$), Fusobacteria (phylum, $P < 0.05$), Deferribacteres (phylum, $P < 0.05$), Verrucomicrobia (phylum, $P = 0.08$), and *Fusobacterium* (genus, $P < 0.05$) in the cecal digesta between egg-laying rate and POS administration.

DISCUSSION

The reproduction performance of breeders can be affected by many factors, including genetics, nutrition, age, management, and environment (Rozenboim et al., 2007; Shi et al., 2020). However, under these same conditions, there are broiler breeders with different reproductive performances. In this study, we also found that, under the same conditions (including genetics, nutrition, age, management, and environment), broiler breeders had different reproductive performances, such as laying rate, egg weight, feed conversion ratio, and qualified egg rate, which is consistent with the previous experiments (Zhao et al., 2019). Pectic oligosaccharide is known as a functional oligosaccharide, which can affect some physiological function of animals. Our recent studies have shown that it increases reproductive performance of pregnant rats (Liu et al., 2020) and improves growth performance of weaned rats and piglets (Mao et al., 2016; Chen et al., 2017). However, the present study showed that dietary POS supplementation only increased egg weight and did not affect laying rate, feed conversion ratio, and qualified egg rate in broiler breeders. These results could reflect the difference between mammals and birds. Generally, the increasing egg weight could mainly be associated with the decrease of laying rate in breeders, but it is also possible that egg weight is associated with gut health. Therefore, we analyzed gut health–related indices.

The nonspecific barrier mechanism is one of the important components that maintain gut function and health (Mao et al., 2011). It contains mucosa epithelial integrity, a tight junction between epithelial cells, and the mucus gel layer. Generally, morphological analysis of the mucosa is usually considered to be evaluation of mucosa epithelial integrity (Potten et al., 1992). The expressions of some transmembrane and nonmembrane proteins, such as ZO, may be used to determine the tight junction between epithelial cells (Laukoetter et al., 2006). Mucins are the main constituent of the mucus gel layer on the intestinal mucosa (Deplancke and

Table 6. The effect of dietary pectic oligosaccharide supplementation on mRNA expressions of tight junction proteins and *mucin 2* in the jejunal mucosa of broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
ZO-1	1.00[b]	1.99[a]	1.26[b]	2.19[a]	0.13	0.17	<0.05	0.84
ZO-2	1.00	0.80	0.92	1.13	0.06	0.30	0.95	0.09
mucin 2	1.00	1.00	0.91	0.88	0.09	0.18	0.39	0.39

[a,b]Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet; ZO, zonula occluden.

Table 7. The effect of dietary pectic oligosaccharide supplementation on mRNA expressions of some cytokines in the jejunal mucosa of broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
IL-1β	1.00	0.66	0.61	0.87	0.13	0.76	0.88	0.29
IL-6	1.00^{c}	1.04^{c}	7.50^{a}	2.70^{b}	0.55	<0.05	<0.05	<0.05
IL-8	1.00	0.98	0.94	1.35	0.18	0.70	0.62	0.59
TNF-α	1.00^{b}	0.91^{b}	1.97^{a}	0.81^{b}	0.08	0.07	<0.05	<0.05
IFN-γ	1.00	1.04	0.99	1.35	0.16	0.65	0.56	0.64

$^{a\text{-}c}$Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; IFN, interferon; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet; TNF, tumor necrosis factor.

Gaskins, 2001). In this study, there are no significant differences in morphology and the mRNA expressions of *ZO-1*, *ZO-2*, and *mucin 2* in the jejunal mucosa between broiler breeders with AELR and LELR. This possibly illustrated that different egg-laying rates did not affect nonspecific gut barrier mechanism in broiler breeders. In addition, we found that POS administration increased *ZO-1* mRNA expression in the jejunal mucosa of broiler breeders and tended to improve morphology and *ZO-2* mRNA expression in the jejunal mucosa of LELR broiler breeders. Although these results of POS regulating nonspecific gut barrier mechanism in broiler breeders were similar to our previous studies on weaned rats and piglets (Mao et al., 2016, 2017b, 2019a), the efficiency of POS in broiler breeders is lower than that in weaned rats and piglets. It was possible that, compared with adult animals, POS administration had better effect on young animals.

Redox balance is important to animal health (including intestinal health), and it is involved in free radical generation and antioxidant capacity (Zheng, 2007). This study showed that concentration of MDA, a kind of lipid peroxide, was increased in the jejunum of LELR broiler breeders, which was relieved by POS administration. In addition, in the present study, dietary POS supplementation enhanced SOD activity, but did not affect total antioxidant capacity in the jejunum of broiler breeders. Thus, MDA generation and POS treatment inhibiting MDA levels should also be derived from other ways. These results had some differences from those of our previous studies on weaned rats and piglets (Mao et al., 2016, 2017b), which further demonstrated that the favorite duration of regulating gut function and health could mainly be the young period.

Inflammation is also one of the factors that affect the generation of free radicals (Zheng, 2007). In further analysis, we measured mRNA expressions of some cytokines (such as *IL-1β*, *IL-6*, *IL-8*, *TNF-α*, and *IFN-γ*) in the jejunal mucosa of these birds. And we found that *IL-6* and *TNF-α* mRNA expressions in the jejunal mucosa of LELR broiler breeders were increased, which was effectively alleviated by supplementing POS in diets. Moreover, the trend of these results was consistent with that of the MDA level in the jejunum, which showed that the results of antioxidant capacity in the present study could mainly be associated with inflammation.

Intestinal microbiota also plays a vital role in physiological function (such as gut health, reproduction) (Salonen and de Vos, 2014). The present study showed that alpha and beta diversity of microbiota in the cecal digesta of AELR broiler breeders is higher than that in the cecal digesta of LELR broiler breeders, and there are some differences of microbiota composition in the cecal digesta between AELR and LELR broiler breeders, which demonstrated that the egg-laying rate could be influenced by gut microbiota. And this study also showed that POS administration did not affect alpha and beta diversity of microbiota in the cecal digesta, but significantly increased abundance of *Phascolarctobacterium* (genus) in cecal digesta of broiler breeders and *Fusobacterium* (genus) in cecal digesta of LELR broiler breeders. *Phascolarctobacterium* is a kind of succinate-consuming bacteria and can prevent *Clostridioides difficile* infection that causes severe gut

Table 8. The effect of dietary pectic oligosaccharide supplementation on alpha diversity of microbiota in the cecal digesta of broiler breeders with different egg-laying rates.

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
Observed species	1,432.00^{a}	1,318.50^{a,b}	1,167.60^{b}	1,148.80^{b}	43.52	<0.05	0.40	0.55
Shannon	7.59^{a,b}	7.63^{a}	7.42^{a,b}	7.37^{b}	0.04	<0.05	0.89	0.55
Chao1	1,585.47^{a}	1,457.55^{a,b}	1,301.46^{b}	1,260.34^{b}	48.46	<0.05	0.34	0.62
ACE	1,593.87^{a}	1,476.74^{a,b}	1,327.75^{a,b}	1,285.25^{b}	48.80	<0.05	0.38	0.68

^{a,b}Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet; ELR, egg-laying rate.

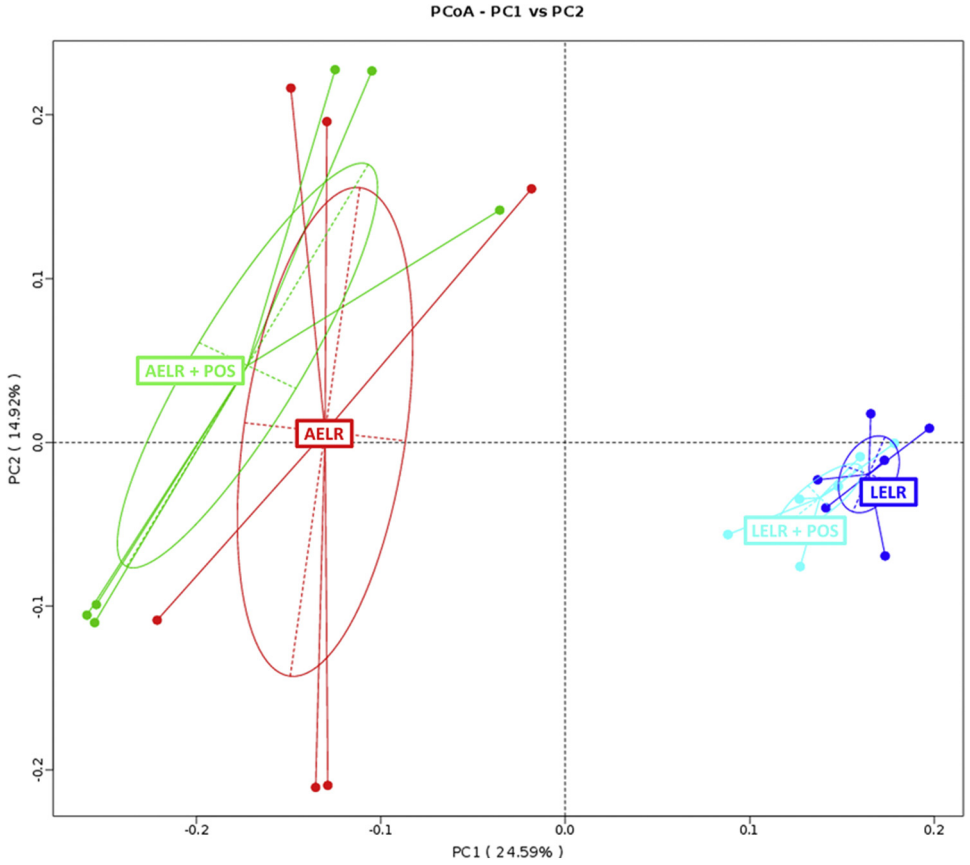

Figure 1. Principal coordinate analysis plot of microbiota in the cecal digesta of broiler breeders with different egg-laying rates based on the unweighted UniFrac metric. Abbreviations: AELR, average egg-laying rate; AELR + POS, average egg-laying rate with dietary pectic oligosaccharide supplementation; LELR, low egg-laying rate; LELR + POS, low egg-laying rate with dietary pectic oligosaccharide supplementation.

inflammation via reduction of use of luminal succinate (Nagao-Kitamoto et al., 2020). Dietary fiber can increase the abundance of *Phascolarctobacterium* in human feces (Hooda et al., 2012). Fusobacteria is a family of obligate anaerobic Gram-negative bacilli, which is the normal microbe of the gastrointestinal tract. It is well known that *Fusobacterium* can affect intestinal health of humans (Arane and Goldman, 2016). Therefore, POS administration could improve gut health via regulation of gut microbiota composition too.

CONCLUSION

Dietary POS supplementation may increase egg weight of LELR broiler breeders. This could be associated with enhancing gut health via the improvement of intestinal barrier function, antioxidant capacity, and microbiota composition. However, the effect of POS administration on gut health in broiler breeders was lower than that in weaned rats and piglets. This could be related to animal species, age, and POS dosage. In

Table 9. The effect of dietary pectic oligosaccharide supplementation on top 10 phylum abundance of microbiota in the cecal digesta of broiler breeders with different egg-laying rates (%).

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
Bacteroidetes	40.90^{b}	44.00^{b}	47.15^{a}	42.33^{b}	0.70	<0.05	0.43	<0.05
Firmicutes	32.96^{b}	32.47^{b}	36.70^{a}	36.13^{a}	0.58	<0.05	0.57	0.97
Fusobacteria	7.45	4.43	3.61	7.02	0.72	0.66	0.89	<0.05
Proteobacteria	8.80^{a}	7.79^{a}	4.25^{b}	4.21^{b}	0.53	<0.05	0.44	0.48
Spirochaetes	1.75	1.94	1.09	1.28	0.19	0.09	0.61	0.99
Kiritimatiellaeota	0.81^{b}	2.46^{a}	0.55^{b}	1.31^{a,b}	0.29	0.19	<0.05	0.41
Euryarchaeota	1.01^{c}	1.55^{b,c}	3.09^{a,b}	3.35^{a}	0.32	<0.05	0.46	0.79
Deferribacteres	0.61^{a}	0.33^{b}	0.38^{a,b}	0.49^{a,b}	0.05	0.72	0.35	<0.05
Verrucomicrobia	0.30	0.19	0.13	0.29	0.04	0.66	0.68	0.08
Actinobacteria	1.75^{a}	1.54^{a}	0.64^{b}	0.70^{b}	0.14	<0.05	0.73	0.53

$^{a\text{-}c}$Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet.

Table 10. The effect of dietary pectic oligosaccharide supplementation on top 6 genus abundances of microbiota in the cecal digesta of broiler breeders with different egg-laying rates (%).

	AELR		LELR			*P* value		
Items	CON	POS	CON	POS	SEM	ELR	POS	ELR × POS
Bacteroides	22.97	24.27	25.29	23.21	0.53	0.56	0.71	0.13
Fusobacterium	7.44	4.43	3.61	7.02	0.72	0.66	0.89	<0.05
Faecalibacterium	5.42^{a}	4.24^{a,b}	3.58^{b}	3.94^{a,b}	0.28	0.05	0.44	0.16
Megamonas	0.30	0.97	0.75	0.67	0.11	0.72	0.19	0.11
Methanobrevibacter	0.70^{b}	1.27^{b}	3.00^{a}	3.25^{a}	0.35	<0.05	0.48	0.77
Phascolarctobacterium	1.38^{b}	1.85^{a,b}	1.52^{a,b}	2.21^{a}	0.13	0.29	<0.05	0.65

^{a,b}Mean values within a row with unlike superscript letters are significantly different ($P < 0.05$).

Abbreviations: AELR, average egg-laying rate; CON, the basal diet; ELR, egg-laying rate; LELR, low egg-laying rate; POS, pectic oligosaccharide–supplemented diet.

future, the relative use of POS in poultry breeding needs to be further researched.

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DISCLOSURES

The authors declare that there are no conflicts of interest.

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