Soybean oligosaccharide, stachyose, and raffinose in broilers diets: effects on odor compound concentration and microbiota in cecal digesta

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ABSTRACT Soybean oligosaccharides have been previously shown to be associated with the production of major odor-causing compounds in broilers, although little is known about the role of stachyose and raffinose, which are key components of soybean oligosaccharide, in broiler cecal microbiota and odor compound production. To this end, soybean oligosaccharide, stachyose, and raffinose were added to the birds' diets to investigate their effects on odor compound production and the microbial community characteristics of the cecum in broilers. A total of 300 one-day-old Arbor Acre broilers with similar initial live weight were randomly allocated into 5 dietary groups with 6 replicates of 10 birds. The diets included soybean meal (positive control), soybean meal-free (negative control), 0.6% soybean oligosaccharide, 0.6% stachyose, or 0.6% raffinose. After a 49-D feeding period, both ceca were aseptically removed postmortem, and the contents were collected and analyzed for skatole, indole, volatile fatty acids, and lactic acid by using high performance liquid chromatography. Bacterial communities were detected by using a high-throughput sequencing platform based on IlluminaMiSeq 2500. Levels of skatole and indole tended to be lower in the dietary supplementation of oligosaccharides. The lowest levels of skatole and indole were observed in the stachyose group ($P < 0.05$), while the highest levels were found in the negative control group ($P < 0.05$). Concentrations of acetic acid and propionic acid in the stachyose group were increased ($P < 0.05$) while those of butyric acid and lactic acid were decreased ($P < 0.05$) compared with the soybean oligosaccharide and raffinose groups. Firmicutes and Bacteroidetes were prevalent in all groups, the proportion of Bacteroidetes was slightly decreased in the stachyose group, and Verrucomicrobia was abundant in the raffinose group ($P > 0.05$). Bacterial genera *Alistipes* and *Parabacteroides* were comparably abundant in the stachyose group, while *Bacteroides*, *Lactobacillus*, and *Akkermansia* were more abundant in the negative control, stachyose, and raffinose groups, respectively. Collectively, these findings demonstrated that dietary oligosaccharide supplementation significantly reduced odor compound production by modulating the cecal microbial community. Compared with soybean oligosaccharide and raffinose, the addition of stachyose into diets may help improve gut fermentation and minimize odor compound generation in broilers.

Key words: skatole, indole, cecal microbiota, oligosaccharide, broiler

INTRODUCTION

A difficult persistent situation occurs between the increasing global population, which is estimated to comprise approximately 9.6 billion individuals by 2050, and the demand for animal protein (Borda-Molina et al., 2018). Correspondingly, intensive poultry production systems have proven economically effective to match the demands of a growing world population. The extreme growth in intensive broiler farming leads to not only high chicken meat yield but also associated adverse environmental problems, such as odor production (Benoît et al., 2001). Strict environmental protection regulations enacted by governments restrict the expansion of broiler production and set limits on the emissions of offensive odors.

The source of odor is mainly due to anaerobic bacterial fermentation of nutrients in the feed in the gut. Many types of odorous compounds have been identified and

classified into 4 main groups: 1) sulfur compounds, 2) phenolic and indolic compounds, 3) volatile fatty acids (**VFAs**), 4) ammonia and amines (Mackie et al., 1998). Among these compounds, indole and skatole (3-methylindole), which are produced from anoxic metabolism of L-tryptophan by microbial degradation, are well-known foul-smelling fecal odorants in mammalian and avian feces (Benoît et al., 2001; Trabue et al., 2010; Tesso et al., 2019). Acetic acid, propionic acid, and butyric acid are the most common VFAs produced from the microbial conversion of dietary residues. A few strategies directed at reducing odorants arising from livestock and poultry breeding have been evaluated, including the modification of diets (Cho et al., 2015; Sharma et al., 2015; Recharla et al., 2017) and addition of oligosaccharides (Rideout et al., 2004; Yang et al., 2016), enzymes (O'Shea et al., 2014; Sharma et al., 2016), organic acids (Claus et al., 2003; Øverland et al., 2008), probiotics, or minerals (Armstrong et al., 2000; Borowski et al., 2017; Tesso et al., 2019).

Our previous studies have demonstrated that the addition of soybean oligosaccharides decreased the concentrations of indole and skatole *in vitro* (Liu et al., 2018c), which may be caused by a decrease in the L-tryptophan degradation rate. Dietary soybean oligosaccharide supplementation also decreased the concentrations of indole and skatole and increased total VFAs in the excreta of broilers *in vivo* (Yang et al., 2016). Moreover, soybean oligosaccharide supplementation can foment the growth of lactic acid bacteria and change the bacterial community structure of broiler cecal contents, which is beneficial for uncultured *Lachnospiraceae* bacterium and *Bacteroides* sp. and contribute to reducing the excreta odor of the broilers (Yang et al., 2016). As the main components of soybean oligosaccharides, stachyose and raffinose were linked to the prevention and inhibition of intestinal pathogenic bacteria colonization and promotion of gut development and health (Ishizuka et al., 2009; Altamimi et al., 2016; Liu et al., 2018b, 2019). Therefore, this study was designed to investigate the effects of soybean oligosaccharide, stachyose, and raffinose in diets of broilers on odor compound production and cecal microbiota.

MATERIALS AND METHODS

Animals

A total of 300 one-day-old Arbor Acre commercial broiler chicks with similar initial live weights were used in this study. The chicks were randomly allocated into 5 treatments with 6 replicates of 10 birds (5 males and 5 females) each and housed in 3-layer cages equipped with a separate feeder and a nipple drinker under a constant 24-h light condition. The room temperature was maintained at 33°C for the first 3 D, after which the temperature was gradually reduced by 3°C each week until reaching 25°C, which was maintained until the end of the 49-D experiment. All procedures involving animals were approved by the Animal Care and Use Committee of Shenyang Agricultural University.

Diets

Diets (in mash form) for the broilers were formulated in accordance with the Arbor Acres management guide (Beijing Arbor Acres Poultry Breeding Co., Ltd.) and shown in Table 1. Soybean oligosaccharide, stachyose, and raffinose products were obtained from Guangzhou YiBaoLai Biotechnology Co., Ltd. (Guangzhou, China) and contained 997.3 g/kg, 853.3 g/kg, and 630.4 g/kg of total sugar (measured values), respectively. Five groups were designated: a group fed a typical corn-soybean meal diet (positive control), a group fed a soybean meal-free diet (negative control), a group each fed 0.6% soybean oligosaccharide, 0.6% stachyose, or 0.6% raffinose as a supplement to the negative control diet on a total sugar basis. The diets were fed in a unlimited manner, and all broilers were given free access to water from nipple drinkers for the experimental period of 49 D.

Sample Collection

Cecal digesta samples of broilers from each replicate (male and female in half per treatment) for skatole, indole, VFAs, and microbial examination were collected on the day of slaughter (49 D). For each broiler chicken, both ceca were taken out, and then the cecal digesta was collected in a tube and stored at −80°C until further analysis.

Skatole and Indole Analysis

The concentrations of skatole and indole in cecal digesta were quantitatively measured by high-performance liquid chromatography (**HPLC**) method as described before (Yang et al., 2016). Briefly, 1 g of cecal digesta and 2 mL of distilled water were added to a 5-mL centrifuge tube, which was homogenized by a tissue homogenizer for 15 s before centrifuging at $3,000 \times g$ for 10 min. Then, 1 mL of supernatant liquid and 2 mL of methyl alcohol were added to a 5-mL centrifuge tube, mixed by a vortex mixer, and then kept at −20°C for 30 min to precipitate particles. After centrifuging at $3,000 \times g$ for 10 min, 1 mL of supernatant liquid was transferred into a 1.5-mL centrifuge tube and then centrifuged at $15,000 \times g$ for 30 min. The collected supernatant liquid was passed through a 0.45-µm filter membrane for the analysis of skatole and indole using an HPLC (1100; Agilent, Wilmington, DE) equipped with a fluorescence detector and a chromatographic column (250 mm $\times$ 4.6 mm $\times$ 5 µm; Dikma, Beijing, China). The mobile phase was the mixture of acetonitrile and distilled water at a ratio of 60:40 (v/v) with a flow rate of 1.0 mL/min. The excitation wavelength and emission wavelength were 263 nm and 358 nm, respectively, and the sample injection volume was 20 µL.

Table 1. Feed ingredients and nutrient composition of experiment diets (air dry basis, g/kg).

	Corn-soybean meal diets		Soybean meal-free diets	
Feed ingredients	Starter (1–21 D post-hatch)	Finisher (22–49 D post-hatch)	Starter (1–21 D post-hatch)	Finisher (22–49 D post-hatch)
Corn	468	511	414	473
Soybean meal	233	169	0.0	0.0
Corn umbilicus pulp	80	50	234	160
Corn protein meal	60	60	187	152
Soybean oil	37	45	35	43
Rice bran	50	80	50	80
Corn DDGS	30	50	30	50
Limestone	12.5	11.8	12.0	11.4
Calcium hydrophosphate	13.5	10.0	14.2	10.5
Sodium chloride	3.0	3.0	2.6	2.7
DL-methionine	3.0	2.5	2.4	2.0
L-lysine	4.9	4.6	10.0	8.4
L-threonine	1.6	1.2	2.2	1.7
L-arginine	1.5	0.8	4.5	3.0
Choline chlotide	0.5	0.5	0.5	0.5
Vitamin mineral premix[1]	1.5	1.5	1.5	1.5
Phytase	0.1	0.1	0.1	0.1
Nutrient composition[2]				
Metabolizable energy (MJ/kg)	12.76	13.21	12.76	13.21
Dry matter	903.5	906.3	901.8	899.6
Crude protein	216.5	196.7	218.2	197.9
Calcium	9.6	8.1	9.5	7.8
Total phosphorus	6.8	6.2	6.9	6.3
Available phosphorus	3.6	3.0	3.6	3.0
Lysine	15.1	13.6	14.6	14.2
Methionie	6.4	5.9	6.3	5.7
Methionie + cystine	9.5	7.9	9.3	8.3

[1]Provided per kg diet: VA, 22,500 IU; VB$_1$, 2.5 mg; VB$_2$, 1.5 mg; VB$_6$, 20 mg; VD$_3$, 5,500 IU; VE$_3$, 35 IU; antioxidant, 0.25 mg; pantothenic acid, 5 mg; folic acid, 2 mg; niacin 75 mg; biotin 0.12 mg; Mn, 60 mg; Fe, 44 mg; Zn, 76.5 mg; Cu, 6.8 mg; and K, 10 mg.
[2]The metabolizable energy and available phosphorus were calculated values, while the others were determined based on triplicate assays.

VFA and Lactic Acid Analyses

VFA and lactic acid analyses were performed by using an HPLC system (1100; Agilent) equipped with a photodiode array detector and a chromatographic column (250 mm $\times$ 4.6 mm $\times$ 5 μm; Dikma). Briefly, 1 g of cecal digesta and 2 mL of distilled water were mixed, homogenized, and centrifuged at 3,000 $\times$ g for 10 min. Then, 1 mL of the supernatant liquid was taken into a 2-mL centrifuge tube containing 0.2 mL of 25% metaphosphoric acid and centrifuged at 15,000 $\times$ g for 10 min. The supernatant liquid was collected and filtered through a 0.45-μm filter membrane to determine the concentrations of VFAs and lactic acid. The mobile phase was phosphate buffer (pH = 2.5) and methyl alcohol at a ratio of 95:5 (v/v) with a flow rate of 1.0 mL/min, and the sample injection volume was 10 μL.

DNA Extraction and PCR Amplification

Subsequently, 200 mg of cecal digesta was weighed and put into a 2-mL sterile centrifuge tube. Total genomic DNA from the cecal digesta samples was extracted using the cetyltrimethylammonium bromide method as previously described (Yang et al., 2016). Bacterial 16S ribosomal DNA genes containing V3-V4 of the variable region were amplified using the 341F and 805R primer pair (F: CCCTACACGACGCTCTTCCGATCTG, R: GACTG-GAGTTCCTTGGCACCCGAGAATTCCA). The amplification conditions of the PCR were followed: one

cycle at 95°C for 3 min; 30 cycles of 30 s at 95°C, 30 s at 50°C, and 60 s at 72°C; and one cycle at 72°C for 7 min.

Bioinformatic Analysis

The cecal microbiota was detected using a second-generation high-throughput sequencing platform based on IlluminaMiSeq 2500.

After sequencing, the primer connector sequences of the raw sequenced reads were discarded, and the paired reads were merged one sample read according to barcode label. Whole sample reads were filtered for quality control and processed to remove nonamplification sequences and chimeric sequences using the Usearch and Uchime software packages (drive5.com; Edgar, 2010; Edgar et al., 2011), respectively, and assembled to obtain operational sequences using the Uclust algorithm (Edgar, 2010). Sets of sequences with $\geq$97% identity were defined as an operational taxonomic unit. We used Ribosomal Database Project classifier software and the Greengenes database (Wang et al., 2007) for species annotation and statistical analyses of the species composition for each sample at the phylum and genus levels. The Shannon, Chao1, and Simpson indices were used to estimate alpha diversity (Qiao et al., 2018).

Statistical Analysis

Data were analyzed using a one-way ANOVA via the software SPSS 17.0 (SPSS Inc., Chicago, IL), and

significant differences among groups were compared using Duncan's multiple comparison tests. Probability values less than 0.05 ($P < 0.05$) were considered for all measured variables.

RESULTS

Odorous Compound Production

As shown in Table 2, the concentration of indole in the negative control group was higher than that in the positive control, soybean oligosaccharide, and raffinose groups ($P < 0.05$), and the lowest indole concentration was observed in the stachyose group. The indole levels in the soybean oligosaccharide, stachyose, and raffinose groups were lower than those in the negative control group by 9, 15, and 9% ($P < 0.05$), respectively. Similarly, the concentration of skatole in the negative control group was higher than that in the other groups ($P < 0.05$) and that in the positive control, soybean oligosaccharide and stachyose were lower than those in the raffinose group ($P < 0.05$). The concentration of skatole in the soybean oligosaccharide, stachyose, and raffinose groups were decreased by 42, 45, and 14%, respectively, compared to the negative control group.

VFA and Lactic Acid Production

As shown in Table 3, the concentrations of acetic acid and propionic acid in the stachyose group were higher than those in the other groups ($P < 0.05$). The highest concentrations of butyric acid and lactic acid were observed in the positive control group ($P < 0.05$). Compared with the negative control, the concentrations of acetic acid in the soybean oligosaccharide, stachyose, and raffinose groups were increased by 114, 180, and 68%, respectively; propionic acid were increased by 551, 783, and 381%, respectively; butyric acid were increased by 68, 33, and 81%; and lactic acid were decreased by 21, 35, and 68%, respectively.

Cecal Microbiota

The microbial 16S rDNA V3-V4 region of 20 cecal digesta samples in 5 treatments were sequenced base on IlluminaMiSeq sequencing technology. Operational taxonomic units were classified according the similarity of 97%. The raw sequence range of each sample was 45,624 to 85,230 reads, the sequence length was 400 to 440 bp after quality control (removal of the barcode, primer, and low-quality sequences), and the range of filtered sequences was 40,330 to 77,896 reads. There was no difference in the number of raw sequences, chimeras, and filtered sequences among groups ($P > 0.05$).

Cecal Microbial Alpha Diversity Analysis

The microbial complexity in the cecum was estimated on the basis of alpha-diversity indices (Chao1 index, Simpson and Shannon index). The Chao1 index was

Table 2. Effects of soybean oligosaccharides, stachyose, and raffinose on the concentrations of skatole and indole in the cecum of broilers (μg/g).

Groups	Skatole	Indole
Positive control	1.1[c]	1.8[b]
Negative control	1.9[a]	2.0[a]
Soybean oligosaccharide	1.1[c]	1.8[b]
Stachyose	1.1[c]	1.7[c]
Raffinose	1.7[b]	1.8[b]
SEM	0.07	0.02

[a-c]Figures with different superscripts within the same column are significantly different ($P < 0.05$).

used to estimate species richness, and index of Shannon and Simpson was used to indicate species diversity. There was no difference in the Chao1 and Simpson indices among groups ($P > 0.05$), although the Shannon index of the positive control was higher than that of the raffinose group ($P < 0.05$) (Figure 1).

Microbial Species Annotation

We annotated the microbial species found in this study. A total of 13 phyla and 66 genera were obtained from the 20 broiler cecal samples. There were 7 phyla with proportions greater than 0.1% (Figure 2): Firmicutes, Bacteroidetes, Verrucomicrobia, Proteobacteria, unclassified, Synergistetes, and Actinobacteria. Firmicutes was the dominant phylum in the cecal microbiota, accounting for 71% of the overall bacteria community, although no significant difference ($P > 0.05$) was observed among groups. Bacteroidetes was the second most abundant (24%) phylum in the cecum, and the average abundance value of both Firmicutes and Bacteroidetes was over 94.6% in all groups. The number of Verrucomicrobia (8.87%) was the highest in the raffinose group, and the number of Proteobacteria (1.38%) in the stachyose group was higher than that of soybean oligosaccharide (0.72%) and raffinose (0.86%) groups. Interestingly, although no Verrucomicrobia was found in the positive control, the unclassified bacteria were overrepresented (1.44%) compared with the other groups.

Figure 3 shows the structure of the bacterial community of all groups in the broiler cecal samples at the genus level. The sequences were more abundant by an unclassified genus with average relative abundance >40% among groups, and the 16 major classified genera across all groups were the *Alistipes*, *Parabacteroides*, *Faecalibacterium*, *Ruminococcus2*, *Bacteroides*, *Clostridium IV*, *Butyricicoccus*, *Akkermansia*, *Subdoligranulum*, *Lactobacillus*, *Butyricimonas*, *Intestinimonas*, *Blautia*, *Pseudoflavonifractor*, *Rumonococcus*, and *Clostridium XIVa*, with relative abundance >1%. The results showed that *Alistipes* was more abundant in the negative control, soybean oligosaccharide, and raffinose groups, whereas *Parabacteroides* was more abundant in the positive control. *Alistipes* and *Parabacteroides* were comparably abundant in the stachyose group ($P > 0.05$). The highest proportion of *Bacteroides* was

Table 3. Effects of soybean oligosaccharides, stachyose, and raffinose on the concentrations of volatile fatty acids and lactic acid in the cecum of broilers (mg/g).

Groups	Acetic acid	Propionic acid	Butyric acid	Latic acid
Positive control	1.4[d]	0.5[d]	1.2[a]	0.8[a]
Negative control	1.2[d]	0.3[d]	0.4[d]	0.7[a,b]
Soybean oligosaccharide	2.6[b]	2.0[b]	0.7[b]	0.5[b]
Stachyose	3.4[a]	2.7[a]	0.6[c]	0.3[c]
Raffinose	2.1[c]	1.5[c]	1.8[b]	0.7[a,b]
SEM	0.15	0.19	1.15	0.04

[a-d]Figures with different superscripts within the same column are significantly different ($P < 0.05$).

observed in the negative control group, and that of *Lactobacillus* in the stachyose group, although these were not different from the other groups ($P > 0.05$). Finally, *Ruminococcus* and *Ruminococcus2* were decreased in the soybean oligosaccharide group and *Akkermansia* and *Pseudoflavonifractor* were increased in the raffinose group.

DISCUSSION

Many attempts have been made to understand the process of odorous compound production and reduce odor emission from livestock and poultry system. Our previous studies have demonstrated that the older the broiler, the higher the odorous compound concentrations (Zhang, 2016). The skatole and indole concentrations in the digesta of intestinal segments from broilers varied and followed the order of the cecum > rectum > ileum (Yang et al., 2019). In addition, the concentrations of skatole and indole in the cecum were significantly higher than those in the rectum and excreta, and the concentrations of odorous compounds (skatole and indole) in the cecum showed positive correlations with those in the excreta (Wang et al., 2016). Therefore, the cecal digesta of broilers were sampled to determine the levels of cecal odorous compounds at the end of feeding trial (49 D old) in this study.

Indole and skatole result from a multistep degradation of L-tryptophan by microbial activity, which are considered to be the contributors to chicken excreta malodor (Roager and Licht, 2018). Skatole is a malodorous

compound that contributes to the characteristic smell of animal fecal material (Liu et al., 2018a). Our previous studies have demonstrated that the *in vitro* addition of soybean oligosaccharide decreased the concentrations of skatole and indole in broiler cecal and rectal microbiota fermentation broth (Liu et al., 2018c). The inclusion of 3.5 to 5.0 g/kg of soybean oligosaccharide to the diet had a decreasing effect on the excreta skatole of broilers (Li, 2018), and except for soybean oligosaccharides, the addition of stachyose, raffinose, and saccharose also decreased the concentrations of skatole in broiler cecal microbial broth (Yang et al., 2017). The results of the study by Myint et al. (2018) suggested that feeding bean husk as a dietary fiber supplement (containing 0.1% stachyose and 0.1% raffinose in carbohydrate) exhibited lower skatole and indole levels in rat cecum. Similarly, the present study indicated that dietary supplementation to a soybean meal–free diet with soybean oligosaccharide, stachyose, and raffinose significantly reduced skatole and indole levels in broiler cecal digesta.

Oligosaccharides are a constituent of dietary fiber that cannot be broken down by the host's digestive enzymes in the small intestine but can be fermented to a large extent by microorganisms in the lower gastrointestinal tract into VFAs, such as acetic acid, propionic acid, and butyric acid (Bedford and Gong, 2018). Soybean oligosaccharides have been demonstrated to contribute to the growth of beneficial bacteria and improve the intestinal microenvironment, thus resulting in the change of VFAs concentrations. Zhou et al. (2012) found that
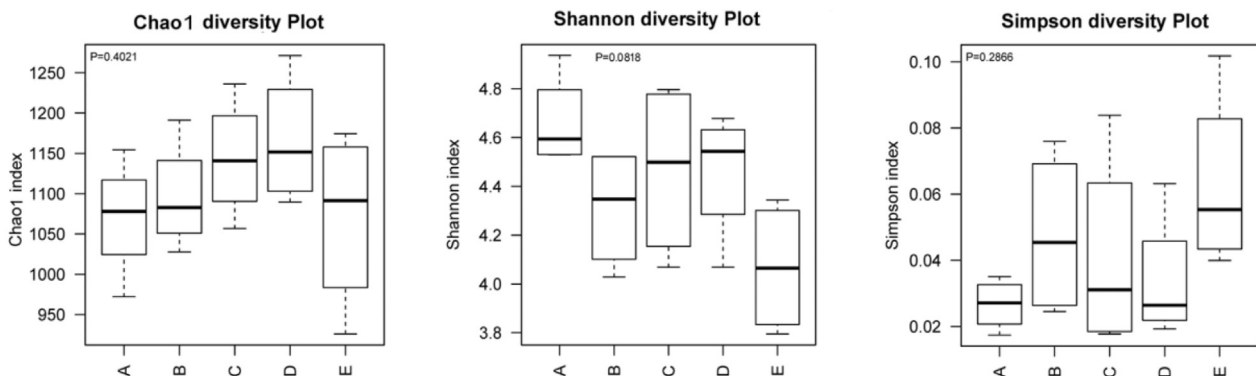

Figure 1. Effects of soybean oligosaccharides, stachyose, and raffinose on the cecal microbiota diversity and richness in broilers. A, B, C, D, and E are the positive control, negative control, soybean oligosaccharide, stachyose, and raffinose groups, respectively.
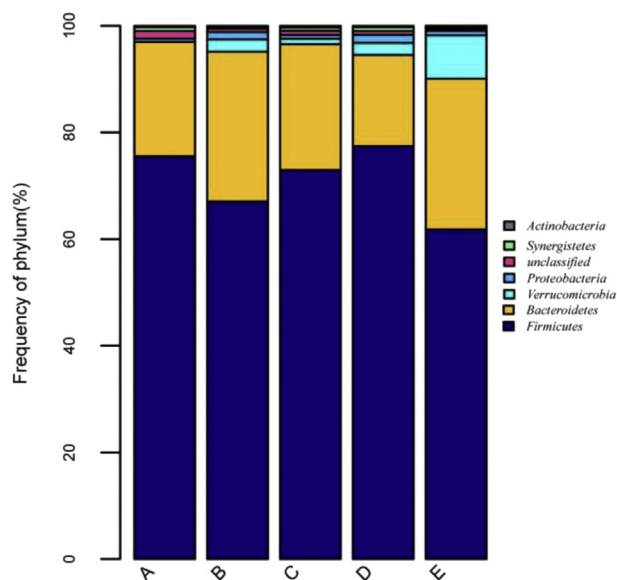
Figure 2. Relative abundance of bacterial phylum in the cecal digesta of broilers. A, B, C, D, and E are the positive control, negative control, soybean oligosaccharide, stachyose, and raffinose groups, respectively.

the *in vitro* addition of 2% soybean oligosaccharides into the fermentation broth of pig cecal digesta increased the concentrations of acetic acid, propionic acid, and butyric acid. Lan et al. (2007) also found that soybean oligosaccharides increased the concentrations of acetic acid, propionic acid, and butyric acid *in vitro* fermentation of broiler cecal contents. Our previous studies have demonstrated that the addition of soybean oligosaccharides increased the acetic acid level *in vitro* and total VFA level *in vivo* (Yang et al., 2016; Liu et al., 2018c). Here,

we found that dietary oligosaccharide supplementation can increase cecal VFAs and decrease lactic acid concentration in broilers, and among oligosaccharides, stachyose was superior to soybean oligosaccharides and raffinose, implying that stachyose may be the key functional component of soybean oligosaccharide.

It is worth mentioning that our preliminary study of the project showed that the growth of broilers at 1 to 49 D of age in the positive control group was the best (owing to the dependence of broiler chickens on soybean meal), followed by the stachyose group, although this was not different from the negative control and the soybean oligosaccharide group. The worst growth of broilers was observed in the raffinose group (unpublished data). These data mean that the birds fed stachyose or soybean oligosaccharides grew well compared with those in the negative control group and had no effect on odorous metabolites.

The gut microbiota of the host plays important roles in absorbing nutrients, enhancing growth and metabolism, protecting against harmful bacteria, and modulating the immune system (Borda-Molina et al., 2018; Rowland et al., 2018). Thus, it is essential to elucidate the link between gut microbiota and odor compound production to understand the internal mechanism. Soybean oligosaccharides have been previously shown to modulate the structure and biomass of broiler cecal microbiota fermentation broth *in vitro*, which decreased the microbiota richness but not microbiota diversity, thus resulting in lower L-tryptophan degradation rate and skatole and indole levels (Liu et al., 2018b). Yang et al. (2016) found that supplementation of soybean oligosaccharides into diets increased the Shannon Wiener index and richness of the
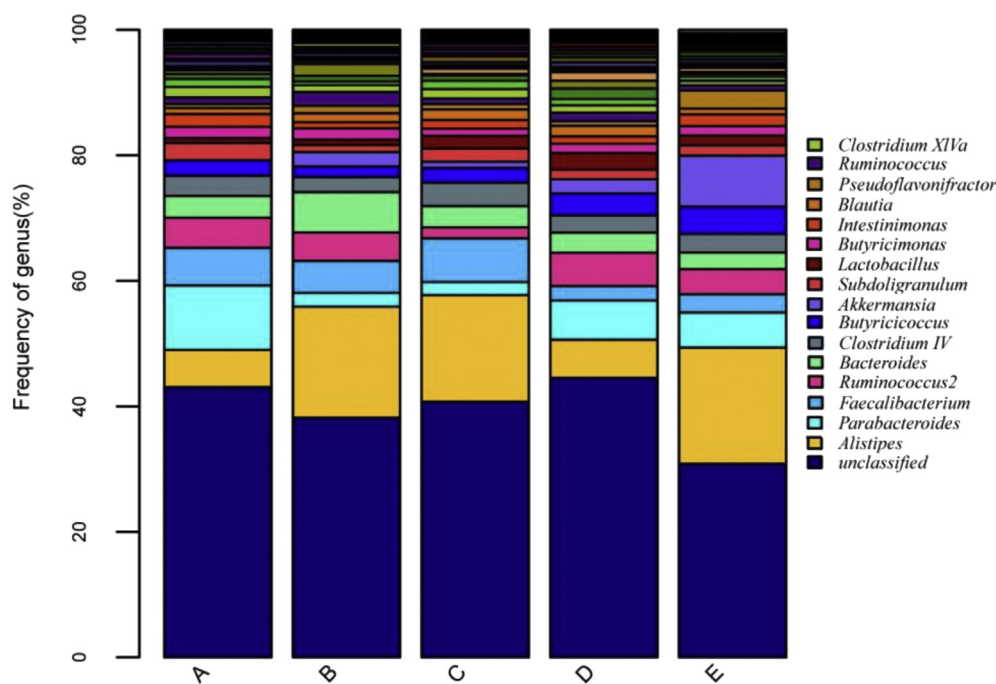

Figure 3. Cecal microbial composition of bacterial genera in broilers. A, B, C, D, and E are the positive control, negative control, soybean oligosaccharide, stachyose, and raffinose groups, respectively.

microbiota, increased the total VFAs, and decreased skatole and indole levels in the cecal digesta of broilers. Zhou et al. (2014) also suggested that the addition of soybean oligosaccharide could improve the balance of cecal microbial community and increase the diversity of intestinal microbiota. In this study, we found that dietary soybean oligosaccharide and stachyose supplementation could modulate the balance of intestinal microbiota to maintain the diversity and richness of cecal microbiota, while raffinose supplementation decreased the diversity of cecal microbiota in broilers.

The cecum is highly dominated by not-yet-characterized bacteria and exhibits the highest concentrations of VFAs (Rowland et al., 2018). Generally, the normal cecal microbiota of chicken are mainly colonized by the phyla Firmicutes, Bacteroides, and Proteobacteria (Borda-Molina et al., 2018). Consistent with this, the present study also showed that Firmicutes and Bacteroides constituted the majority of the cecal bacteria, which were associated with carbohydrate and protein metabolism. In addition, the relative abundance of Bacteroides in the stachyose group was lower than that in the other groups, indicative of its role in the process of deamination and decarboxylation of tryptophan to produce malodorous compounds, such as skatole (Cho et al., 2015). Conversely, the higher Proteobacteria abundance in the stachyose group than the soybean oligosaccharide and raffinose groups may account for the relatively lower skatole and indole levels. Noticeably, raffinose may serve as a preferential fermentation substrate for Verrucomicrobia, which was abundant in the raffinose group.

At the genus level, our study showed that *Alistipes*, *Parabacteroides*, and *Faecalibacterium* were the main groups in the cecum, which was different from other studies in which the cecum was dominated by *Acinetobacter*, *Bacteroides*, *Streptococcus*, *Clostridium*, and *Lactobacillus* (Borda-Molina et al., 2018), although they were similar to the study by Xiao et al. (2017) in which it was indicated that *Alistipes*, *Ruminococcus*, and *Faecalibacterium* were the dominant bacteria genera in the cecum. *Alistipes* and *Bacteroides* can metabolize tryptophan to indole-3-lactate and then to indole and skatole in animal faces (Cho et al., 2015), which is consistent with the observation in this study that the higher abundance of *Alistipes* and *Bacteroides* in the negative control was along with the higher concentrations of skatole and indole, although it was completely opposite to the findings in the stachyose group. *Lactobacillus*, a widely used probiotic, inhibits the growth and colonization of pathogenic bacteria via the production of organic acids (such as lactic acid) or bacteriocins (Caly et al., 2015). Nevertheless, in this study, the abundance of *Lactobacillus* in the stachyose group did not increase the lactic acid levels, although the concentration of lactic acid in the stachyose group was lower than that in other groups. Presumably, lactic acid may be a fermentation substrate to be used and converted into propionate or butyrate by other bacteria, thus leading to lower levels in the cecal digesta

(Rowland et al., 2018). The abundance of *Akkermansia*, which is a genus in the phylum Verrucomicrobia, in the raffinose group further suggested that it was related to the degradation of raffinose. In general, our findings clearly indicate an evident change in bacterial community, microbial fermentation, and odor compound production of the cecum in broilers after dietary oligosaccharide supplementation.

In conclusion, the results from this study indicate that the addition of oligosaccharides into diets affects odor compound production and the bacterial community composition. Supplementation of stachyose rather than soybean oligosaccharide or raffinose reduced odor compound production and increased acetic acid and propionic acid levels of the cecum in broilers. The microbiome analysis revealed that the bacterial community is related to the production of skatole, indole, VFAs, and lactic acid. Compared with soybean oligosaccharides and raffinose, stachyose can preferably improve cecal fermentation and help minimize odor compound generation from broiler cecum. We suggest that the results of this study might contribute to the reduction of odor compounds in broilers.

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