Soybean Oligosaccharides Supplementation Attenuates the Fecal Odor Compounds by Modulate the Cecal Microbiota of Broilers

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Research

Keywords: 16S rRNA, soybean oligosaccharides, chlortetracycline, odor compounds, cecal microbiota, broilers

DOI: https://doi.org/10.21203/rs.3.rs-35493/v1

Abstract

Background: Abatement of odor emissions in poultry production is very important for the quality and safety in poultry industries and benefit to the environment.

Methods: This study was conducted to evaluate the effects of the dietary supplementation of different levels soybean oligosaccharides (SBO) in comparison with chlortetracycline (CHL) on major odor-causing compound in excreta and cecal microbiota of broiler chickens. One-day-old broiler chickens were assigned to 6 treatments with 6 replicate pens (10 birds/pen) for the 42-day experiment, including, the negative control (NC) fed a basal diet, the positive control (PC) fed a basal diet with CHL, and the basal diet with SBO at 0.5, 2.0, 3.5, and 5.0 g/kg, respectively. Fresh excreta was sampled for analysis odor compounds by high performance liquid chromatography. Cecum content was collected to analyze the cecal microbiota by 16S rRNA sequencing.

Results: The excreta indole concentration of broilers fed 2.0, 3.5 and 5.0 g/kg SBO and CHL diets were significantly decreased ($P < 0.01$) compared to NC. Excreta skatole concentration ($P < 0.001$) and pH ($P < 0.05$) were decreased by SBO and CHL. Formate concentration of birds fed 3.5 and 5.0 g/kg SBO diets were higher than that of birds fed other diets ($P < 0.001$). The acetate concentration ($P = 0.003$) were increased in birds fed 3.5 g/kg SBO diet. Deep sequencing 16S rRNA revealed that the composition of the cecal microbial digesta slightly or significantly changed by the supplementation of SBO or CHL. SBO decreased the abundance of *Bacteroides*, *Bilophila*, *and Escherichia*, which were related to indole and skatole concentration of excreta. While CHL had strong tendency to enrich *Ruminococcus* and reduce *Rikenella*.

Conclusion: These results indicated that supplementation of dietary SBO was beneficial in attenuating the concentration of odor causing compounds and impact the composition cecal microbiota of broilers.

Background

Broiler chickens are an indispensable source of animal protein for human beings, more than 65 billion chickens are produced annually in the word [1]. Meanwhile, broilers production also brings a huge amount of odor substances. Abatement of odor emissions in poultry production is very important for the quality and safety in poultry industries and benefit to the environment [2, 3]. The odor compounds, mainly including skatole (3-methylindole), indoles and volatile organic compounds, are produced by the microbial degradation of the nutrient substrates in the large intestine[4, 5]. These substances could cause the decline in performance, meat quality and welfare of broilers. Billions of microbes inhabit in the large intestine and act as mediators of these reactions of broilers. The component of diet is thought to be the key factor in influencing the composition and metabolic activity of the intestinal microbiota, and further effects in the production of odor compounds [6–8]. Therefore, altering the ingredient of the diet may be possible to reduce odor emission from the source of odor [9, 10].

Soybean oligosaccharides (SBO) are one group of non-digestible carbohydrates, which are extracted from soybean seeds, primarily consist of stachyose, raffinose, and sucrose [11, 12]. While the exact mode of action remains elusive, it is usually considered that dietary SBO could be selectively fermented by some types of intestinal bacteria in the large intestine, resulting in decrease the contents of the major odor-causing compounds and better growth performance of animals [3, 13]. Several studies have shown a link between the cecal microbiota and odor-causing compounds of animals by dietary SBO. For example, diets with SBO significantly changed the population of *Bifidobacteria*, *Lactobacillus* and *E. coli*, and decreased the content fecal NH_3 in broilers [14]. SBO supplementation modifies the intestinal ecosystem in weaned piglets and has potentially beneficial effects on the gut [15]. Similar study in mice revealed that administration of SBO improved the numbers of benefical intestinal microbes [16]. In vitro studies have revealed that SBO can improve the gut microbiota balance in colon and modulate its metabolism in pigs [7], changed in the intestinal microbiota and reduced the production of odor compounds in broilers [17, 18]. Antibiotics work primarily by reshaping the intestinal microbiota [19], which is known as a unique ecosystem to play a crucial role in host health and metabolism [20–22]. However, the change and relationship between the compounds of major odor compounds and the intestinal microbiota are not elucidated by adding dietary SBO. It also remains unknown whether SBO and antibiotic modulate the intestinal microbiota in distinct manners.

We hypothesized that the mode of action of different levels of dietary SOB or antibiotic effect on odor-causing compounds content in excreta and occurs through the composition of cecum microbiota of broilers. Therefore, it is necessary to better understand how cecal bacterial communities react to these feed additives and suitable level of addition. In these study, we used high-throughput sequencing of the V4 region of the bacterial 16S rRNA gene to assess the alter of structure cecal bacterial community and the change of major odor compounds of excreta in broiler chickens after fed either a commercial diet free of antibiotics (NC), the same basal diet supplemented with a sub-therapeutic level of chlortetracycline (PC), or the basal diet supplemented with 0.5 g/kg to 5.0 g/kg of SBO (0.5SBO, 2.0SBO, 3.5SBO, or 5.0SBO). The new light on their produce mechanism and may allow SBO as targeted manipulation of the intestinal microbiota to reduce odor compounds and improve animal health and productivity in the future.

Methods

Experimental design, birds and diets

A total of 360 day-of-hatch commercial mixed sex Archer Abor^{+}-hybird broiler chicks were obtained from a Commercial Hatchery (Shenyang, China) and randomly assigned to one of six dietary treatments with 6 replicates (n = 10) per treatment in a completely randomized block design. All birds were fed a non-medicated common commercial-type broiler starter (d 0–21) and grower (d 22 to 42) corn-soybean meal basal diets and offered water ad libitum. Detailed information about the basal diets is summarized in Additional file 1. The six treatment groups were as follows: negative control (NC), basal diet was no additives; positive control (PC), the same basal diet supplemented with 40 mg/kg chlortetracycline (CHL),

and SBO at 0.5 g/kg (0.5SBO), 2.0 g/kg (2.0SBO), 3.5 g/kg, (3.5SBO), and 5.0 g/kg (5.0SBO), respectively. The basal diets were commercially manufactured feed which formulated to meet NRC (1994) requirements without antibiotics. The SBO was extracted from soybean meal, and the main ingredient was stachyose, with purity of 80.92% [23].

The chickens were raised in cages pens in an environmentally controlled broiler house. The ambient temperature on 1 d was 35 ± 2 ℃, and was decreased 3 ℃ per week until it reached 26 ℃, thereafter, the temperature was kept constant until 42 d. The light was provided 24 h constant for the entire period of the experiment.

Sampling procedures, determination of odor compounds and VFAs

On d 40, 41 and 42, fresh excreta were sampled from each replicate group using plastic trays below each cages within 2 min after excretion. The dropped fresh excreta were collected from the tray and transferred into plastic tubes. The total amount of collected excreta samples from the same replicate were pooled stored in a freezer at − 20℃ for skatole and indole analyses. The pH of excreta was measured using the pH meter (PB-10, Sartorius, Goettingen, Germany). Concentrations of indole, skatole, VFAs (formic, acetate, propionate, and butyrate) and lactate in the excreta were measured using HPLC equipment (model Agilent 1100, Agilent Technologies, Santa Clara, CA) as described Yang et al. [3, 8].

On d 42, one bird per replicate of each treatment was randomly selected, euthanized by cervical dislocation. The contents of ceca were collected under sterile conditions by dissection and pooled two contents by treatment. Samples were immediately frozen in liquid nitrogen and were stored at -80 ℃ for further microbiota analysis through 16S rRNA sequencing.

DNA isolation

The microbial DNA was isolated from cecal content using a QIAamp Fecal DNA Isolation Kits (Qiagen Co., Ltd., Beijing, China) according to the manufacturer's instructions. The quantity and quality of extracted DNA was checked by agarose gel electrophoresis (1.5%) and Nanodrop spectrophotometer (Nyxor Biotech, Paris, France) analysis.

PCR amplification and sequencing

The following primers were applied for the amplification of the variable V4 region of the bacterial 16S rRNA gene: 515F 5′-barcode-GTGCCAGCMGCCGCGGTAA-3′and 806R: 5′-GGACTACHVGGGTWTCTAAT-3′, where the barcode is an 8-base sequence unique to each sample. PCRs were carried out in triplicate 50 μl reactions containing 0.5 μmol L^{-1} forward and reverse primers, 5 μl tenfold reaction buffer, 250 nmol L^{-1}

dNTP, 0.2 μl FastPfu Polymerase and 20 ng template DNA. Thermal cycling consisted of 3 min initial denaturation at 95 ℃, followed by 30 cycles at 95 ℃ for 30 s, 50 ℃ for 30 s, and 72 ℃ for 60 s, and a final extension at 72 ℃ for 7 min. About 400–450 bp fragment of PCR products was obtained and purified with GenJET Gel Extraction Kit (Thermo Scientific Co., USA).

Sequencing libraries were generated using NEB Next® Ultra™ DNA Library Prep Kit (New England Biolabs Co., US) following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit@2.0 Fluorometer (Thermo Scientific Co., US) and Agilen Bioanalyzer 2100 system. At last, samples were sequenced on an Illumina MiSeq 2500 instrument using a 250 bp paired-end reads protocol at the Mymbio Tech Company of Beijing. Paired-end sequencing with dual index reads was performed with automated cluster generation.

Bioinformatic analysis

Paired-end reads from the original fragments were merged using FLASH version 1.2.7 [24]. 16S rRNA reads were decoded based on sample specific barcodes and processed to remove low quality reads data. The quality average score of reads was above 20, joining together the sequences and removed the sequences shorter less than 100 bp reads using Mothur software to filer data. Chimeras and error sequences of optimized data were removed using QIME (version 1.8.0) [25] software package by clustering data into operational taxonomic units (OTUs) for species classification under 0.97 similarity [26, 27]. Rarefaction analysis and good's coverage were determined to quantify the coverage and sampling effort, and statistic the abundance information of each OUT in samples. Collector's curves for richness observations (ACE and Chao1) and Shannon diversity index were calculated. Beta diversity analysis was performed using UniFrac to compare the results of principal coordinate analysis (PCoA) using the community ecology package R-forge and generate PCoA figures. Besides, using the Number Cruncher Statistical System software (NCSS 20017; Kaysville, UT, USA), by the unweighted pair-group (UPGMA) method and Manhattan distance with no scaling, double hierarchical analysis was conducted [28].

Statistical analysis

Data were analyzed by one-way analysis of variance protocol using SPSS 19.0 (Statistical Package for Social Science; SPSS Inc., Chicago). Replicates were considered the experimental units for data of odor compounds and VFAs. Differences between the means were compared using LSD multiple range tests, and statistical significance was set at $P < 0.05$ level. For microbial data, means separation was via four nonorthogonal contrasts of (i) negative control treatment vs. others, (ii) negative control treatment vs. SBO treatments, (iii) positive control treatment vs. others, (iv) positive control treatment vs. SBO treatments. Pearson correlation coefficients (r) were used to evaluate the relationship between odor compounds and genera.

Results

Effect of in-feed SBO on odor compounds and VFAs in excreta

Table 1
Effect of soybean oligosaccharides on indole (ng/g), skatole (ng/g) concentration, and pH in excreta of broilers during excreta collection.

Item	Dietary Treatments[d]						Statistics	
	NC	PC	0.5SBO	2.0SBO	3.5SBO	5.0SBO	SEM[e]	*P*-value
Indole	111.07[a]	60.58[bc]	87.85[ab]	40.49[c]	21.71[c]	37.92[c]	8.040	0.003
Skatole	34.23[a]	23.90[cd]	28.18[b]	22.42[d]	18.68[e]	26.23[bc]	0.964	< 0.001
pH	6.53[a]	6.49[ab]	6.29[abc]	6.21[abc]	6.12[c]	6.30[abc]	0.043	0.023

[a, b, c]Means in a column within treatment grouping without a common superscript letter differ ($P < 0.05$).

[d]NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SOB: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

[e]SEM, standard error of the mean.

The effect of dietary supplementation of different levels SBO on the concentration of skatole and indole, and pH in excreta of broilers on final days are presented in Table 1. The treatments supplementation of SBO decreased indole and skatole of excreta in broiler chickens compared to the NC treatment ($P < 0.05$). The indole ($P = 0.003$) concentration was significant decreased when supplementation of SBO at 2.0, 3.5 and 5.0 g/kg compared to NC, with the average 70% lower than the NC; while there is no significant differences with PC. The skatole concentrations ($P < 0.001$) were significantly lower when supplementation of SBO or CHL than NC, and it was lowest when SBO supplementation at 3.5 g/kg, with less 45.4% and 12.8% than NC and PC, respectively.

VFAs and lactate data are shown for excreta in broiler chickens fed dietary supplements on the final days in Table 2. The birds fed 3.5 and 5.0 g/kg SBO has higher formate than that of the other groups ($P < 0.001$). While feeding birds with diets containing 3.5 g/kg SBO increased acetate level in excreta compared to other treatments ($P = 0.003$). The propionate, butyrate and lactate level of excreta were not affected by the dietary treatments ($P > 0.05$).

Table 2
Effect of soybean oligosaccharides on VFAs and lactate concentrations in excreta of broilers (mg/g)

Item	Dietary Treatments[c]						Statistics	
	NC	PC	0.5SBO	2.0SBO	3.5SBO	5.0SBO	SEM[d]	*P*-value
Formate	0.187[b]	0.176[b]	0.277[b]	0.300[b]	0.561[a]	0.542[a]	0.033	< 0.001
Acetate	0.237[b]	0.223[b]	0.244[b]	0.258[b]	0.293[a]	0.256[b]	0.006	0.003
Propionate	0.256	0.224	0.227	0.244	0.237	0.259	0.022	0.999
Butyrate	0.229	0.140	0.136	0.258	0.212	0.186	0.017	0.327
Lactate	4.108	4.016	4.565	4.378	4.249	4.058	0.188	0.964

[a, b]Means in a column within treatment grouping without a common superscript letter differ ($P < 0.05$).

[c]NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

[d]SEM, standard error of the mean.

Effect of in-feed SBO on the cecal microtiota diversity

Broiler chicks were fed a non-antibiotics corn-soybean basal diet supplemented without or with different levels of SBO and antibiotic for six weeks before collection of cecal content samples for each treatment. Following bacterial DNA isolation and sequencing of the V4 region of the 16S rRNA gene. A total of 4 479 533 raw sequences reads were obtained, and 3 420 000 high-quality sequences were used for further analysis, with an average of 190 000 sequences per sample. There was detected 9 821 difference phylotypes among all samples for OUTs at distance (species level). At OUT level, rarefaction curves was performed and indicated that there were sufficient sequences for sampling to detect the diversity of majority bacteria. The estimators of community richness (ACE and Chao), diversity (Simpson and Shannon index) and good's coverage are shown in Table 3. There was no significant differences in the OUT, ACE, Chao1, Simpson, Shannon, and good's coverage indices in the dietary treatments, but there was a trend significant for OUT, ACE, and the Chao1 ($P < 0.10$). OUT, ACE, and the Chao1 were more for NC compared with others ($P = 0.090$, 0.064, and 0.099), and ACE, and the Chao1were more for NC compared with SBO treatments ($P = 0.070$ and 0.100).

Table 3
The bacterial diversity analysis of samples in different treatment of broilers cecum contents

Item	Dietary Treatments[a]						Statistics	
	NC	PC	0.5SBO	2.0SBO	3.5SBO	5.0SBO	SEM[b]	Contrast[c] (*P*-value)
OTU	2266	1761	1841	1862	1989	1742	84.2	1(0.090)
ACE	2856	2287	2271	2282	2485	2169	102	1(0.064), 2(0.070)
Chao 1	2749	2276	2281	2262	2456	2183	95.8	1(0.099), 2(0.100)
Simpson	0.975	0.945	0.913	0.919	0.907	0.958	0.012	
Shannon	6.87	6.17	6.29	6.28	6.32	6.71	0.180	
good's coverage	0.997	0.997	0.997	0.998	0.997	0.998	0.0001	

[a]NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

[b]SEM, standard error of the mean.

[c]1 = NC vs. others; 2 = NC vs. SBO treatments ($0.05 < P < 0.1$).

Using the Bray-Curtis and Jaccard indices determined β-diversity to reveal the differences in cacal microbiota composition among individual treatments. While there was no obvious segregation of the microbiota that birds fed different diets based on the Bray-Curtis index (Fig. 1A), NC and PC groups were relatively separated from all other groups using the Jaccard index (Fig. 1B).

Effect of in-feed SBO on the bacterial phylum of cecal microbiota

On the classification basis of sequences from the cecal samples, 7 different phyla were identified at the phylum level, which distributed across all the treatment groups (Fig. 2). The others, the remaining phyla with sequence frequencies of < 1%, were considered low abundance. The pyrosequencing results showed

that *Bacterodetes* (43.16–54.74%) and *Firmicutes* (39.62–51.70%) were the dominant phyla for cecal content, accounting for more than 90% (90.57–94.86%) of the total bacterial abundance in broiler chickens fed dietary supplements on d 42 (Additional file 2). The percentage of *Proteobacteria* showed higher for NC than for PC and supplementation SBO diets ($P = 0.049$) (Additional file 2). The abundance of *Tenericutes* was slightly less for the mean of NC or PC compared with SBO diets ($P = 0.101$ and $P = 0.100$, respectively). Feeding birds with diet PC decreased *Actinobacteria* compared to containing SBO diets and NC ($P = 0.023$) or only SBO diets ($P = 0.026$). There were no significant differences between the experimental treatments in the abundance of *Bacteroidetes* ($P = 0.916$), *Firmicutes* ($P = 0.908$), *Unknown* ($P = 0.629$), *Cyanobacteria* ($P = 0.208$) or others ($P = 0.676$).

Effect of in-feed SBO on the bacterial genus of cecal microbiota

At the genus level, the sequences from samples corresponded to 177 genera. The double hierachal cluster analysis on the top 50 most abundant taxa (Fig. 3) indicated that the proportion of unknown flora was the highest (56.73–75.98%) in groups, and other dominant genus included those of the taxa *Bacteroides* (1.47–12.33%), *Ruminococcus* (2.74–4.61%), P*hascolacrctobacterium* (0.18–5.99%), *Rikenella* (0.65–6.96%), *Oscillospira* (1.96–3.89%), *Faecalibacterium* (1.36–5.05%), *AF12* (0.52–2.40%), *Helicobacter* (0.34–2.49%), while the relative abundance of other taxa was below 2%.

The unknown bacteria was less for the mean of NC diet compared with others ($P = 0.047$) (Fig. 4; Additional file 3). Compared with NC, supplementation of PC and SBO ($P = 0.006$) or supplementation of SBO ($P = 0.009$) showed lower percentage of *Bcteroides* (Additional file 3), and lowest was observed at 3.5 mg/kg SBO (Fig. 4). The abundance of *Ruminococcus* was greater for PC than for the birds fed SBO and NC or SBO ($P = 0.028$ and $P = 0.024$, respectively), the mean of fed PC was significant greater than the birds fed SBO at 0.5, 3.5, and 5.0 mg/kg. The percentage of *Rikenella* was less for PC than for other treatments ($P = 0.048$). The *Escherichia* was greater for NC vs. others ($P = 0.026$) or SBO treatments ($P = 0.030$). *Bilophila* was greater for NC vs. others ($P = 0.063$) or SBO treatments ($P = 0.071$), which were close to significant level. There were higher abundance of *Faecalibacterium* for supplementation of SBO levels of 2.0, 3.5 and 5.0 mg/kg, and higher *Lactobacillus* for 0.5, 2.0 mg/kg SBO levels compared to fed NC or PC, although no significant difference among the experimental treatments.

The correlation coefficient between the skatole levels of excreta and bacteria at genus in cecum of broilers

As shown by Table 4, the relationships among odor compounds and cecal digesta microbiota were observed in SBO and control diets of broilers. Indole was highly correlated with *Bilophila* ($P < 0.1$), while skatole was highly related to *Bacteroides* ($P < 0.1$), *Bilophila* ($P < 0.05$), and *Escherichia* ($P < 0.1$). There were high r (positive) for acetate versus *Faecalibacterium* ($P < 0.05$). Propionate was significantly

correlated with Unknown (negative), *Bacteroides*, and *Rikenella* ($P < 0.05$), and lactate was highly correlated with *Lactobacillus* ($P < 0.05$).

Table 4
The correlation coefficient between the odor compound levels of excreta and bacteria at genus in cecum of broilers

Item[a]	Unk	*Bac*	*Rum*	*Rik*	*Fae*	*Bil*	*Lac*	*Esc*
Indole	-0.059	0.493	0.007	-0.104	-0.641	0.751*	-0.017	0.628
Skatole	-0.376	0.744*	-0.049	0.295	-0.536	0.861**	-0.270	0.800*
Formate	-0.384	-0.192	-0.530	0.564	0.699	-0.382	-0.220	-0.281
Acetate	-0.257	-0.234	-0.595	0.224	0.908**	-0.336	0.097	-0.288
Propionate	-0.908**	0.858**	-0.182	0.880**	0.397	0.590	-0.550	0.709
Butyrate	-0.498	0.541	-0.062	0.253	0.743	0.301	0.004	0.382
Lactate	0.507	-0.319	-0.646	-0.358	0.020	-0.303	0.902**	-0.433
pH	-0.131	0.470	0.582	-0.035	-0.707	0.654	-0.453	0.628

[a]Unk = Unknown; *Bac* = *Bacteroides; Rum* = *Ruminococcus; Rik* = *Rikenella; Fae* = *Faecalibacterium; Bil* = *Bilophila; Lac* = *Lactobacillus; Esc* = *Escherichia*.

*$P < 0.1$; **$P < 0.05$.

Discussion

Odor emission from chicken excreta is produced mainly by the microbial degradation the substrates in the cecum of broilers, which is a serious environmental problem in broilers industry. Strategies to mitigate emissions are needed. Among the strategies to reduce odor and gas emissions in the broiler industry, dietary supplementation of feed additives including SBO are becoming an accepted strategy because of their beneficial effects against intestinal pathogenic microbes and reduces the production of odor compounds. The aim of this study was to evaluate the effects of different levels of dietary SBO and CHL on odor-causing compounds in excreta and the composition of cecum microbiota of broilers.

At the end of the experiment, birds fed SBO has indole reduced by 21–80% and impairment of skatole by 33–64% compared with negative treatment. The value of skatole was decreased 22% between 3.5SBO treatment and antibiotic treatment. Also, 3.5 g/kg SBO supplementation decreased the pH, increased acetate in excreta compared with both control treatments, while 3.5 g/kg and 5.0 g/kg SBO had higher formate than that both control treatments. The produced short chain fat acids from fermented

indigestible residues by the abundant microorganisms present in the ceca. Oligosaccharides are able to enter into the ceca in generous amounts and being fermented there by microflora [24]. Our former study has shown similar results [3], when supplementing 1.25 g/kg SBO in broiler decreased indole, skatole and the pH in broiler excreta. Additionally, it has been observed that SBO could decreased the concentration of indole and skatole, and pH value by intestinal microbiota of broilers in vitro [18].

Overall, in this study, the results demonstrated that the dietary supplementation with SBO might be a useful strategy to attenuate the production of odor in broilers. SBO supplementation decreased the concentration of indole, skatole and pH, and enhance the concentration of part of organic acid of excreta in broilers. The results of correlation analysis further indicated that the odor compounds were associated with intestinal microbial community. Broilers fed SBO and CHL had some alterations in the alpha and beta diversity, though non-significant, which indices of the cecal microbiota and these modifications promoted relevant changes of the microbial community structure.

In the early animal trials and in vitro studies, we used the molecular technique of denaturing gradient gel electrophoresis revealed a shift in the microbiota composition [3, 8, 18]. However, this technique lack the depth and precision to reveal specific changes in bacterial composition. Subsequently, the method next-generation sequencing of the bacterial 16S rRNA gene could offer more information on specific changes in certain bacterial populations. However, there is scarce reports about using high-throughput sequencing study the changes on microbial communities of cecum by supplementation SBO in broilers. The approach of high-throughput sequencing provided a much accurate and in-depth estimation of cecal microbial diversity in broilers. Therefore, the results of present study provided the information of the ecology of cecal bacterial communities. In line with the previously reports on cecal microbiota of broilers [1, 30], it was dominated by *Bacteroidetes* and *Firmicutes* at the phylum level, regardless of diet. The metabolism favours the fermentation of cellulose and stach etc in the cecum, results in higher microbial diversity and dominance of the saccarolytic and anaerobic order of *Bacteroides* and *Clostridiales* [1]. The most abundant genera detected in this study were unclassified, *Bacteroides*, *Ruminococcus*, *Phascolacrctobacterium*, *Rikenella*, *Oscillospira*, *Faecalibacterium*, and the unclassifed bacteria was the most. The results were quite similar with those obtained by Wen et al. and Zhu [1, 30]. Bjerrum et al. [30] reported that bacterial strains closely related to *Faecalibacterium prausnitzii* were dominant in the ceca.

Based on the phylogenetic diversity of bacterial communities and number of OTUs, SBO and CHL did not change the chicken cecal bacterial community membership, but the treatments significantly altered relative abundance of certain taxa in the cecum. This observation is in accordance with the study of Zhu et al. [30] who reported that SBO addition (0.6%) altered the chicken cecal microbiota. When added frucotooligosaccharide in broiler diets, the total number of anaerobes and *Lactobacilli* in the ceca increased while the number of *E. Coli* decreased [32]. Similar results were observed by Baurhoo et al by mannanoligosaccharide [33]. In present study, *Bacteroides, Bilophila* and *Escherichia* were significantly decreased in the cecum by SBO (Fig. 4). While *Ruminococcus* (Fig. 4), *Lachnospiraceae* and *Coriobacteriaceae* (Additional file 4) were enriched, *Rikenella* (Fig. 4) appeared to be diminished by chlortetracycline. These results are consistent with earlier observations that in-feed antibiotic

preferentially enriched butyrate-producing bacteria [19], SBO inhibited the growth of pathogenic microbes, and reduced the production of odor compounds [3, 14, 30]. Previous studies have found that supplementation of broiler diets with a mixture of chlortetracycline and other antibiotic increased both *Ruminococcaceae* and *Lachnospiraceae* [34]. It is known that both *Lachnospiraceae* and *Ruminococcaceae* produce butyrate [19, 35, 36].

The most dramatic effect of SBO supplementation is differential reduction of the members of four most dominant bacterial families (*Lachnospiraceae*, *Bacteroidaceae*, *Desulfovibrionaceae*,and *Enterobacteriaceae*) in the chicken cecum (Additional file 4). SBO increased the population of a group of lactic acid bacteria in vitro, genera *Lactobacillus*, *Pediococcus*, *Weissella*, and *Leuconostoc* in the cecal contents of young broiler chickens [17]. In broilers, supplementation of 1.25 g/kg SBO showed a higher cecal bacterial diversity, along with lower excreta indole and skatole production, it also increased the excreta total VFA concentration and decreased the pH value, when compared with that of the broilers fed the control diet [3]. Dietary supplementation with SBO increased the diversity of intestinal microflora and elevated the numbers of some presumably beneficial intestinal bacteria, (eg, *Bifidobacterium sp*, *Faecalibacterium prausnitzii*, *Fusobacterium prausnitzii*, and *Roseburia*), also increased the concentration of short-chain fatty acid in the intestinal lumen, and reduced the numbers of bacteria with pathogenic potential (eg, *Escherichia coli*, *Clostridium*, and *Streptococcus*) and the concentration of several protein-derived catabolites (eg, isobutyrate, isovalerate, and ammonia) in piglets[15]. In contrast, Ma et al. [16] described that intragastric administration of 4.0 g/kg body weight SBO significantly enhanced the proliferation of *Bifidobacteria* and lactic bacteria, and increased numbers of *Enterococci* and decreased numbers of *Clostridium perfringens* in the fecal contents of mouse.

Two lactic acid bacterial genera including *Lactobacillus* and *Enterococcus* were differentially regulated by SBO among the abundant OTUs in the cecum, however, no statistical differences were observed on the relative abundance of these bacterial genera between treatments, which may be related with the adding levels of SBO (Additional file 3). Lactic acid bacteria provide a myriad of beneficial effects to the host, and are widely used as probiotics in animal production [37, 38]. It is interesting to note that *Lactobacillus* was upregulated by 0.5, 2.0, and 3.5 g/kg SBO, while the *Enterococcus* was obviously reduced in response to 0.5, 2.0, and 3.5 g/kg SBO. An upregulation in the *Lactobacillus* abundance is consistent with earlier observations that SBO administration was associated with population of the *Lactobacillus* species [17, 39]. The lactate concentration in excreta was significantly positive correlation with *Lactobacillus* in the cecum (Table 4). Both *Lactobacillus spp.* and acetate production in the cecum of chickens by xylo-oligosaccharides treated diets have been shown to be increased, may promote intestinal health [40].The abundance of *Enterococcus* was reduced by lower levels of SBO (Additional file 3). *Enterobacter aerogenes* has been showed to be responsible for the degradation of L-tryptophan to skatole in cecum of broilers [18]. A lone *Proteobacteria* (*Escherichia*) was also reduced by all levels of SBO and CHL, and significantly positive correlation with the levels of skatole in excreta, which is in agreement of earlier reports of a downregulation of *Escherichia* in response to in-feed SBO[16, 18]. *Escherichia* is a typical harmful bacterium in the intestinal tract that is involved in the degradation of tryptophan to indole [41]. These results collectively suggest that SBO has a strong tendency improve the intestinal microbial

balance [3] by favouring a quick proliferation of beneficial strains and inhibiting the growth of pathogenic microbes [7] and then contributes to reduce the production of odor compounds [18, 42].

Our results show that a higher positive correlation for acetate versus *Faecalibacterium*, which is a bacterial stain known to ferment mono-oligosaccharides and oligosaccharides into butyrate and lactate [31]. The intestinal metabolite skatole is generated by decarboxylation of indoleacetic acid by *Bacteroides spp*. and *Clostridium spp*. [43]. Indole formation is converted from tryptophan via the action of the enzyme tryptophanase, which is expressed in many Gram-positive bacterial species including *Escherichia coli, Clostridium spp.* and *Bacteroides spp.* [44–46]. Our results show that a positive correlation for indole versus *Bilophila*, skatole versus *Bacteroides*, *Bilophila* and *Escherichia*, which is in agreement of earlier report of *Uncultured Lachnospiraceae* bacterium and *Bacteroides sp.* are associated with the production of major odor-causing compounds in the excreta of broilers [3].

Indole and skatole are mainly produced by the microbial degradation of several substrates of cecum of broilers. The present study demonstrated that supplementation of broilers dietary SBO significantly reduced the production of odor compounds in excreta, and this effect was associated with cecal microbiota composition, in comparison to its un-supplemented control counterpart, and these partly similar to those of CHL individuals.

Conclusions

In summary, our data indicates that dietary SBO supplementation modulated the relative abundance of some specific bacteria without changing the whole microbial structure. Decreased the relative abundance of *Bacteroides*, *Rikenella*, and *Escherichia* bacteria in cecum and elevated fecal acetate concentration in chickens fed SBO might be an intestinal skatole mitigating attribute and may contribute to ameliorate the producing of odor-causing compound during chicken production.

Abbreviations

SBO: Soybean oligosaccharides; CHL: Chlortetracycline; NC: Negative control; PC: Positive control; NRC: National research council; VFA: Volatile fatty acid; HPLC: High performance liquid chromatography; OTUs: Operational taxonomic units; PCoA: Principal coordinate analysis; SEM: Standard error of the mean

Declarations

Acknowledgment

The authors are grateful to the staff of the Department of Animal Science and Technology of the Shenyang Agricultural University for their valuable assistance in conducting the experiments.

Funding

The National Natural Science Foundation of China (No. 31772618 and 31372328) supported this study.

Availability of data and materials

All data generated or analyzed during this study are available from the corresponding author by request. The datasets supporting the conclusions of this article are included in the article.

Authors' Contributions

Guiqin Yang conceived and designed the experiments; Haiying Liu, Xin Li and Weiguo Dong performed the experiments. Haiying Liu, Xin Li, Xin Zhu analyzed and interpreted the data. Haiying Liu and Li Xin, and Guiqin Yang drafted and revised the manuscript. All authors contributed to data interpretation and approved the final version of the manuscript.

Ethics approval and consent to participate

All animal trials were conducted in accordance with the Institutional Animal Care and Use Committee of Shenyang Agricultural University.

Consent for publication

Not applicable.

Competing interests

All authors approved the submission of this manuscript and declare no conflicts of interest. The manuscript has not been previously published and is not under consideration for publication elsewhere. The agencies that funded this research had no role in the study design, analysis, or writing of this article.

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Conflicts of Interest

The authors declare no conflict of interest.

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Figures

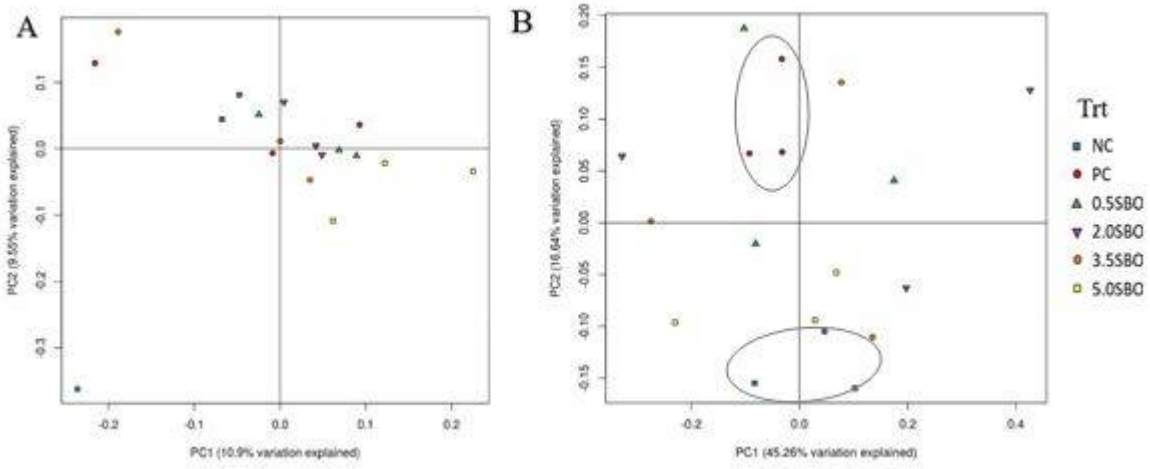

Figure 1

The β-diversity of cecal microbiota of 42-day-old broilers. Principal components (PCs) 1 and 2 accounted for 10.9 and 9.55% (A), for 45.26 and 16.64% of the variance (B), respectively. NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

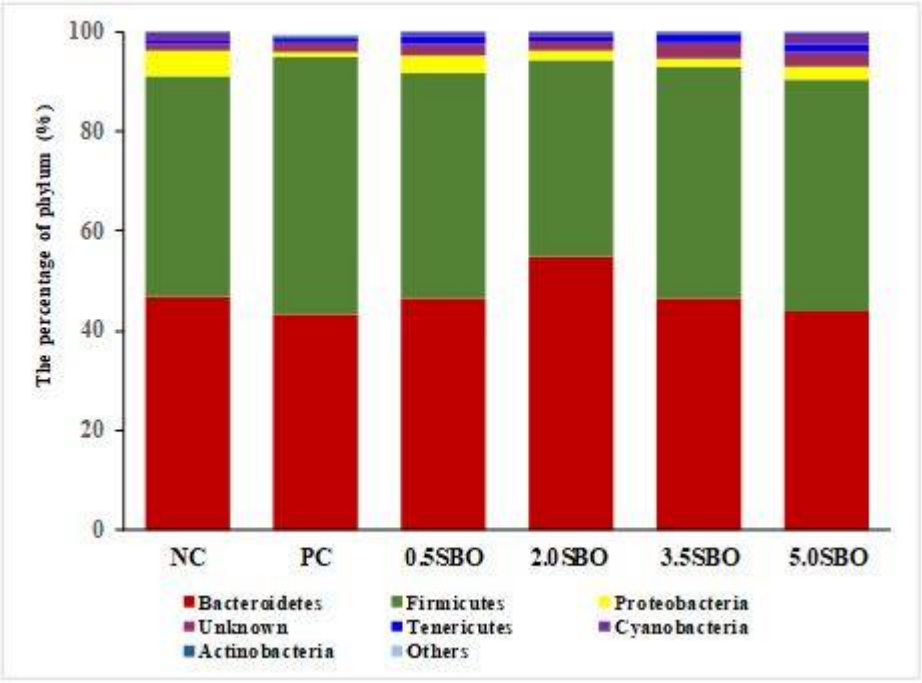

Figure 2

The microbiome compositions in cecum at phylum level for 16S rRNA sequences in cecal digesta of broilers fed with different levels soybean oligosaccharides and control diets. NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

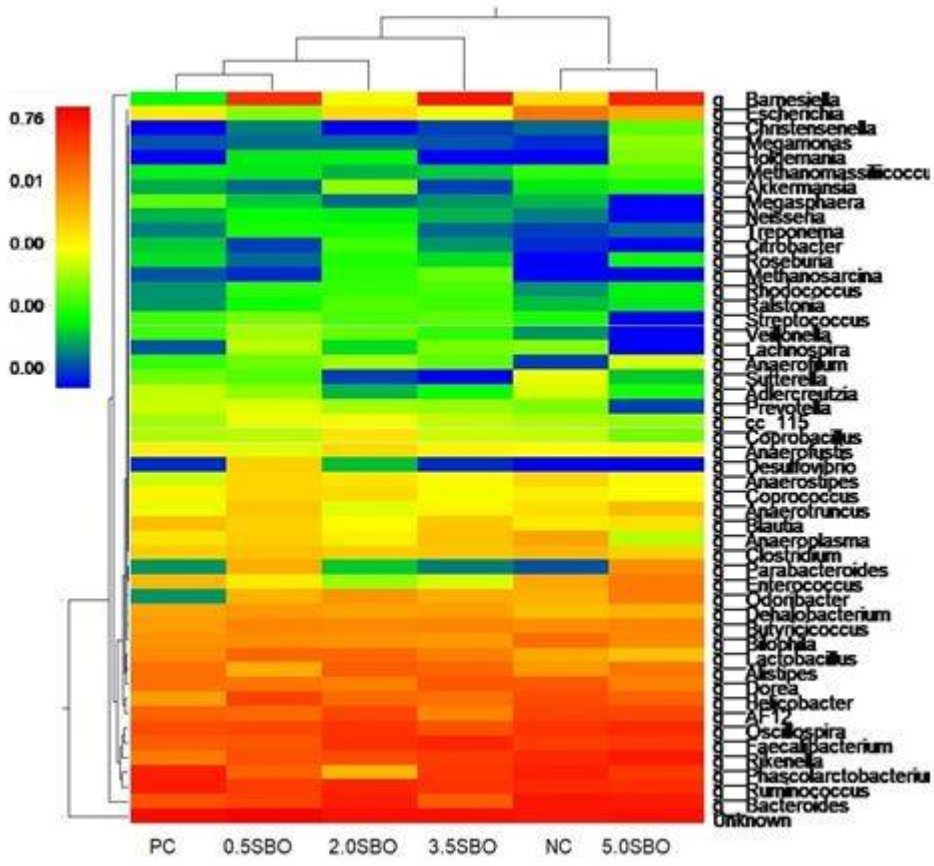

Figure 3

Heat map of relative abundance of the predominant genera in different level SBO groups. Both rows and columns were clustered using the Euclidean distance and average linkage. NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

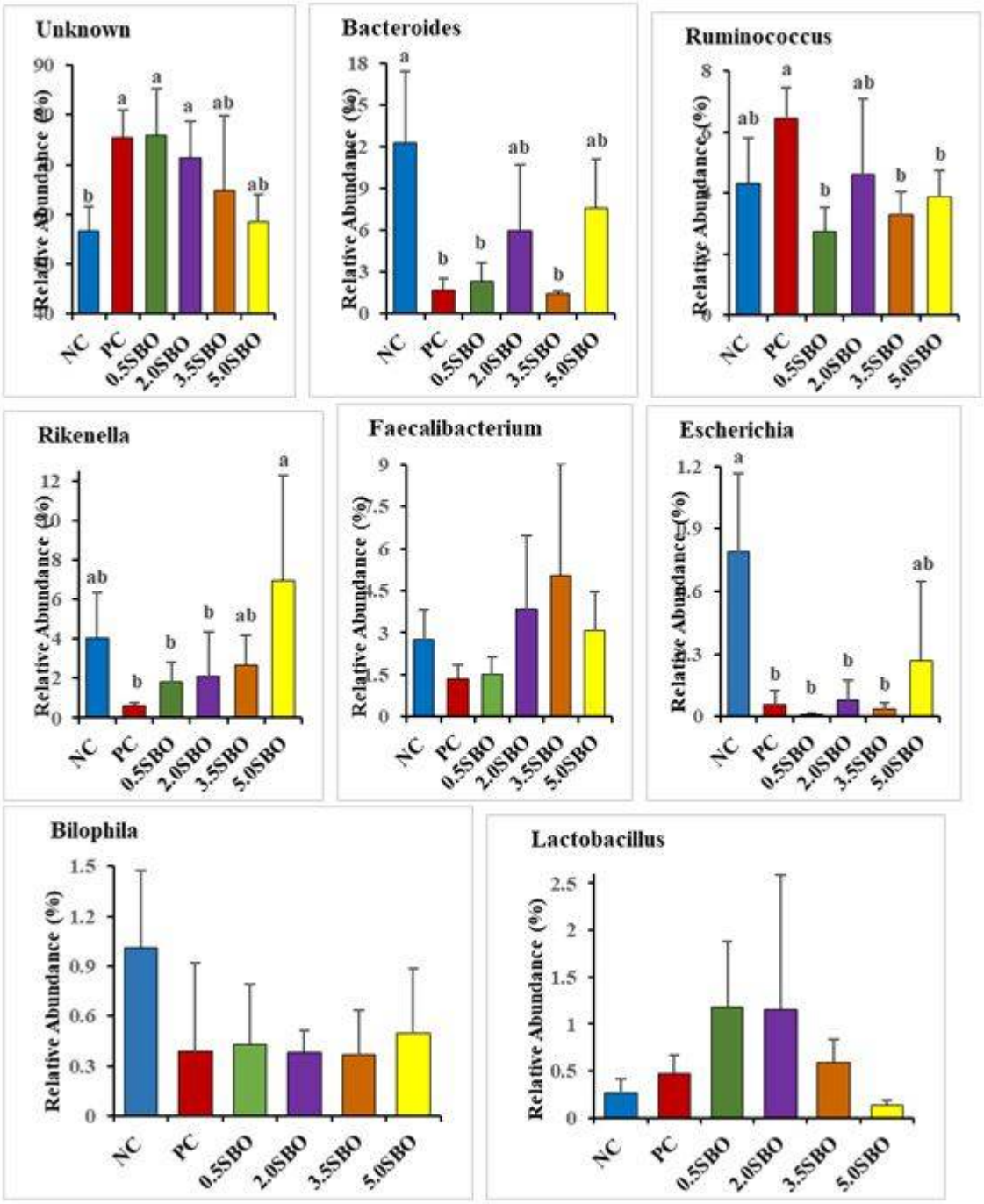

Figure 4

Relative abundance of the predominant genera in different level SBO groups. Values are presented as the mean ± SEM (n = 3 per group), with the treatments not sharing a common letter considered significantly different ($p < 0.05$). NC, negative control; PC, positive control, NC with 40 mg/kg chlortetracycline; 0.5SBO, 2.0SBO, 3.5SBO, and 5.0SBO: NC with 0.5, 2.0, 3.5, and 5.0 g/kg soybean oligosaccharides respectively.

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