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Utilization of Shredded Steam-Exploded Pine Particles as a Dietary Ingredient to Modify Cecal Microbiota in Broilers

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Abstract: Sawdust and wood shavings are the major industrial waste from wood and its related industries. Steam-explosion treatment enhances the utilizable fiber fraction in pinewood particles. This study investigated the effects of adding up to 2% of steam-exploded pine particles (SPPs) in diets on the growth performance and cecal microbiome of broilers. On the 8th day of age, 216 Ross 308 broilers were allocated to three treatments of 72 broilers/group, with 12 replicates of 6 broilers each, to receive 0%, 1%, and 2% SPPs in their diets. The experimental period was from the 8th to 28th day of age. The parameters recorded included growth performance, relative organ weight (duodenum, jejunum, ileum, liver, and spleen), absolute organ length (duodenum, jejunum, ileum, and cecum), blood biochemicals (glucose, total protein, triglyceride, and cholesterol), and microbial analysis from cecum samples. Growth performance parameters, such as the average daily gain, average feed intake, feed conversion ratio, organ weight, length, and blood biochemical parameters, were not influenced by dietary supplementation of SPP. The abundance of fibrolytic bacterial genera, such as *Mediterraneibacter* and *Anaerobutyricum*, was increased in 2% SPP-supplemented chickens. An unknown bacterial genus was also enhanced in 2% SPP-supplemented diets related to the family of fiber-degrading bacteria and needs further investigation. In conclusion, 2% SPP can be supplemented in chicken diets as a source of fiber from wood industry-related waste without having any harmful effects on broiler chickens.

Keywords: wood industry waste; steam-exploded pine particles; growth performance; metagenome; broilers

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1. Introduction

Poultry production holds a respectful position to meet the ever-increasing demand for edible protein. Continuous improvement in the genetic strains in meat-type chicken has reduced the marketable age. The extensively competitive environment of the livestock industry puts pressure to minimize the cost of production. Feed formulation and additives have greatly improved the production performances and feed efficiency of modern broiler strains [1]. Nutritional programming is among the most favorable methods to produce maximum weight with minimal expenditure. Feed cost is the major expenditure for rearing meat-type chicken [2]. Precision in nutrition may help overcome this issue. Supplementation of economical products having sufficient availability and arising from unused stuff can be beneficial.

Industrial and agricultural byproducts accumulate as waste and result in a problem for handling. Sawdust and shavings are the most underutilized waste of the wood industry [3]. They are mostly used as bedding in the poultry industry. There is a considerable possibility to utilize this waste as a fraction in animal feed. Multiple problems can be solved by utilizing these wastes, such as reducing the accumulation of waste and the cost of animal feed. In particular, waste arising from wood sources are rich in fibers. The soluble fibers are suitable for fermentation and short-chain fatty acid (SCFA) production in the gut. The primary concern in utilizing wood waste is its high insoluble fiber content, which negatively influences nutrient digestibility and energy intake by diluting the nutrients [4]. Inconsistent results have been obtained by using different insoluble fiber sources in chicken feed [5] that encourage the possibility to explore the enrichment of insoluble fibers in wood waste. Recent development in technology has shown a way to modulate the insoluble fiber contents [6]. An additional benefit may be the utilization of mechanical techniques, such as heat treatment, for the pretreatment of wood powder to modulate the dietary fiber content. Steam explosion, for instance, could be used to depolymerize hemicellulose in an ecofriendly and economical way [7]. Furthermore, it may help transform the lignocellulosic biomass structure to be efficiently used as feed additives [6,8,9]. If used properly, dietary fibers can act as a prebiotic product that can modulate growth performance by favoring the growth of beneficial microbes in the intestine. The potential action of prebiotic products is as a substrate for facilitating the fermentation process, enhancing luminal osmosis and modulating immunity by macrophage stimulation and SCFAs production, which may behave as an antagonist to foodborne pathogens [10].

The primary role of the gastrointestinal tract (GIT) is to digest and assimilate the nutrients of food. The non-digestible fibers present in undigested food are transported to the ceca after passing through the upper GIT track [11]. The ceca in chickens are the site for the microorganisms containing both favorable and pathogenic microorganisms [12]. Cecal microbiota has a direct connection with feed efficiency, which may further be correlated with growth performances in chickens [13]. For instance, *Bacteroides* and *Lactobacillus* are positively correlated with feed efficiency and growth performances, respectively [13,14]. Evaluation of the cecum microbiota will provide a deep insight into how the microbial population is altered due to shredded steam-exploded pine particle (SPP) supplementation and how the growth performance in chickens is modulated.

It is essential to reduce both industrial wastes by their utilization and feed cost in livestock production due to its highly competitive nature. Considering the impact of non-digestible fibers on the chicken gut, the present experiment was conducted to evaluate the effects of increasing the dose of SPP as the source of fiber on broilers' growth performances and gut microbiota. We hypothesized that SPP can be supplemented to the poultry feed by up to 2% without having any adverse effects.

2. Materials and Methods

The present experiment was conducted at the animal research facility of the Gyeongsang National University, Korea. All the experimental procedures were approved by the Animal Ethics Committee of the Gyeongsang National University (GNU-200916-C0057).

2.1. Production of Shredded Steam-Exploded Pine Particles

The pinewood was collected from the nearby forest of Jinju, Korea, and chopped into pieces to produce a wood chip of approximately 2 cm × 2 cm × 0.5 cm in size. The wood chips were then exploded with steam of approximately 200 °C for 11.5 min. The spray-dried pine particles thus produced were then stored at 20.0 °C until use. The content of crude fiber in 0%, 1%, and 2% diets was 2.70%, 3.12%, and 4.11% for the grower, and 2.77%, 3.43%, and 4.13% for the finisher, respectively (Table 1). The insoluble, soluble, and total fiber contents of the SPP were 83.9%, 0.6%, and 84.5%, respectively.

Table 1. Formula and chemical composition of the experimental diets.

Items	Grower			Finisher		
	0%	1%	2%	0%	1%	2%
Ingredients (%)						
Yellow corn, ground	57.1	55.2	53.2	63.3	61.2	59.1
Soybean meal	33.3	33.5	33.8	28.7	29.0	29.3
Corn gluten meal	3.0	3.0	3.0	2.5	2.5	2.5
Limestone	1.6	1.6	1.5	1.5	1.5	1.5
Tallow	3.6	4.4	5.1	3.3	4.0	4.8
Mono-dicalcium phosphate	0.42	0.43	0.44	0.05	0.05	0.05
Salt	0.3	0.3	0.3	0.3	0.3	0.3
Choline chloride (50%)	0.07	0.07	0.07	0.03	0.03	0.03
DL-methionine hydroxy analogue, 88%	0.15	0.15	0.15	0.10	0.10	0.10
L-Lysine	0.17	0.16	0.15	0.04	0.05	0.05
SPP	0.0	1.0	2.0	0.0	1.0	2.0
Vitamin mixture [1]	0.2	0.2	0.2	0.2	0.2	0.2
Mineral mixture [2]	0.1	0.1	0.1	0.1	0.1	0.1
Calculated values						
TMEn, kcal/kg	3103	3102	3102	3150	3150	3150
Crude protein, %	21.03	21.02	21.03	19.02	19.03	19.02
Calcium, %	0.91	0.92	0.91	0.82	0.81	0.82
Available Phosphorus, %	0.42	0.42	0.43	0.36	0.36	0.36
Lysine, %	1.15	1.15	1.15	1.01	1.01	1.01
Methionine + Cystine, %	0.84	0.83	0.83	0.71	0.72	0.71
Analyzed values (%)						
Moisture	10.98	11.19	10.91	9.87	9.92	10.33
Crude protein	20.59	22.03	20.94	19.69	18.81	19.03
Ether extract	6.97	8.07	7.69	6.92	7.46	7.49
Crude fiber	2.70	3.12	4.11	2.77	3.43	4.13
Ash	4.32	4.34	4.31	4.80	3.87	3.72

[1] Vitamin mixture provided the following nutrients per kg: vitamin A, 40,000,000 IU; vitamin D3, 8,000,000 IU; vitamin E, 10,000 IU; vitamin K3, 4000 mg; vitamin B1, 4000 mg; vitamin B2, 12,000 mg; vitamin B6, 6000 mg; vitamin B12, 20,000 µg; pantothenic acid, 20,000 mg; folic acid, 2000 mg; nicotinic acid, 60,000 mg. [2] Mineral mixture provided the following nutrients per kg: Fe, 30,000 mg; Zn, 25,000 mg; Mn, 20,000 mg; Co, 150 mg; Cu, 5000 mg; Ca, 250 mg; Se, 100 mg. Abbreviations: SPPs, shredded steam-exploded pine particles; TMEn, true metabolizable energy.

2.2. Experimental Birds and Housing

One-day-old mixed-sex Ross 308 broiler chicks (n = 260) were obtained from a local hatchery and raised at the recommended temperature with continuous lighting, as recommended by the ROSS 308 Management Breeding Guide. In the present study, broiler chicks as hatched without sexing were used as in previous studies [15]. The temperature in the first three days was maintained at 34 °C and gradually decreased to 22 °C by the end of the experiment. Chicks were reared for the first seven days in a housing facility containing 20 chicks in each cage with continuous lighting with nipple-type waterers and were fed ad libitum with a commercial starter diet. On the 8th day, individual chicks were weighed and assigned (n = 216) to one of three different treatment groups containing 12 replicates in each treatment and 6 birds in each cage. The experimental diets contained 0, 1%, and 2% SPP replacing corn in their feed ingredients (Table 1). The grower feed was provided from the 8th day to the 21st day of age, while finisher feed was provided from the 22nd day to the 28th day of age. All the birds were distributed equally in two identical adjoining rooms with similar sizes and conditions. Both the rooms were similar in dimensions and to normalize the room effects, all three treatments were equally distributed in each room with a similar number of birds. There was no difference between the two rooms in terms of size, temperature, light, ventilation rate, etc. Birds were reared until the 28th day of age. Feed and water were provided ad libitum throughout the experiment. Average daily feed intake

(ADFI), average daily gain (ADG), and feed conversion ratio (FCR) were calculated based on weekly body weight and feed intake in each cage and mortality was recorded daily if happened.

2.3. Collection of Samples and Measurements

At the end of the experiment, on 28 days of age, eight birds from each group were randomly selected and euthanized in a carbon dioxide chamber. Blood samples were immediately collected through cardiac puncture in a heparinized vacutainer for further plasma processing following the procedures of Kang et al. [16]. Briefly, blood was centrifuged at 2000× *g* at 4 °C for 10 min for harvesting of plasma, which was then immediately stored at −20 °C until use.

Internal organs were dissected, weighed, and their weights were recorded and expressed relative to their body weight. The length of the duodenum, jejunum, ileum, and cecum was also recorded and presented as the absolute length. Both pouches of cecum from each bird were first tied at the ileocecum junction, excised carefully, measured, and snap-frozen in liquid nitrogen for microbiome analysis.

Plasma concentrations of glucose, total protein, triglycerides, and total cholesterol were measured using a VetTest Chemistry Analyzer (IDEXX Co., Ltd., Westbrook, ME, USA) with a dry-slide technology as described previously [16] following the manual instructions [17]. Briefly, slides for glucose, total protein, triglyceride, and total cholesterol were purchased (IDEXX Co., Ltd., Westbrook, ME, USA) and installed in the analyzer. The plasma sample (70 µL) was loaded by an automated pipettor through a disposable plastic pipette tip and the reading was analyzed. Results were then recorded from the analyzer screen.

2.4. Extraction of DNA and Metagenome Analysis

The extraction of cecal microbiome DNA was performed using a DNeasyPowerSoil Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. The quantification of DNA was done using Quant-IT PicoGreen (Invitrogen, Waltham, MA, USA). A 16 S metagenomic sequencing library was constructed to estimate the cecum DNA metagenome. Herculase II Fusion DNA Polymerase Nextera XT Index Kit V2 (Illumina, San Diego, CA, USA) was used for this purpose following the manufacturer's instructions. The sequencing was performed using the Illumina platform at Macrogen, Inc. (Seoul, Korea). In the initial screening for quality control, three samples (one from each treatment group) were removed. For all the remaining 21 samples, FASTQ files were created and each sample underwent quality profiling, adapter trimming, and read filtering using the fastp program [18]. FLASH (1.2.11) software was used to assemble the sequences [19]. Sequences with less than 400 bp and more than 500 bp were removed. Determination of operational taxonomic units (OTUs) was performed by the CD-HIT-EST program using de novo clustering with a 97% sequence identity cutoff [20]. A taxonomic similarity check was performed using BLAST+ (v2.9.0) program [21]. The NCBI 16 S Microbial database was used as a reference database. Identical coverage less than 85% was identified as not defined. The microbial communities and taxonomic information were analyzed using QIIME (v1.9) software. Microbial diversity and homogeneity were evaluated through Chao1, Shannon, Goods Coverage, and Inverse Simpson Indices. A rarefaction curve was used to present the alpha-diversity, while unweighted/weighted UniFrac distances were used to present beta diversity.

2.5. Statistical Analysis

The data for growth performances were analyzed using a general linear model (GLM) procedure of one-way ANOVA using an IBM SPSS statistics software package 25.0 (IBM software, Chicago, IL, USA). Before analysis, Shapiro–Wilk and Levene's tests were used to evaluate the normality of distributions and equality of variances. Duncan's multiple range test was used to test whether significance existed. Each cage was treated as an experimental unit for growth performances. For microbial analysis, alpha-diversity (community

richness and diversity) and taxonomic analysis (phylum and genus) were done using the Kruskal–Wallis test and adjusted with Bonferroni correction. Beta diversity analysis is presented as principal coordinate analysis (PCoA) based on unweighted and weighted Unifrac differences. Evaluation of the bacterial phylum correlation was performed using Spearman's correlation. All data were expressed as means ± SEM. Differences were considered statistically significant at $p < 0.05$ unless otherwise stated. Graphs were drawn using Prism-GraphPad software.

3. Results

Table 2 presents the effects of different concentrations of dietary SPP supplementation on growth performance parameters from 2 to 4 weeks. ADG, ADFI, and FCR were similar ($p > 0.05$) and not affected by dietary SPP supplementation in chickens. However, the final weight was 2.94% higher in the 2% SPP group, and the last week's FCR was 1.19% lower compared to the control.

Table 2. Effects of dietary shredded steam-exploded pine particle supplementation on the growth performance of broiler chickens.

Parameters	0%	1%	2%	pSEM	*p*-Values
Body weight (g) at day 8	202.9	202.8	202.9	0.05	0.747
Body weight (g) at day 28	1458.4	1467.2	1502.7	19.29	0.624
Day 8–14					
ADG (g)	38.9	38.1	39.9	0.70	0.601
ADFI (g)	45.3	43.8	46.0	0.76	0.505
FCR (kg feed/kg body weight)	1.15	1.17	1.16	0.01	0.811
Day 15–21					
ADG (g)	64.4	64.4	66.5	1.24	0.736
ADFI (g)	92.2	89.9	93.4	1.61	0.682
FCR (kg feed/kg body weight)	1.41	1.42	1.41	0.01	0.967
Day 22–28					
ADG (g)	76.1	77.7	79.3	1.19	0.545
ADFI (g)	128.9	127.4	130.7	2.00	0.810
FCR (kg feed/kg body weight)	1.67	1.66	1.65	0.01	0.690

Chickens were fed with diets containing 0% (control), 1%, and 2% shredded steam-exploded pine particles (SPPs) from the 8th day to the 28th day of age. Data show mean ($n = 12$). Abbreviations: ADG: average daily gain; ADFI: average daily feed intake; FCR: feed conversion ratio; pSEM: pooled standard error of the mean.

Table 3 presents the effect of SPP supplementation on the weight of the digestive organs in the chickens. The weight of the digestive organs, such as the duodenum, jejunum, ileum, liver, and spleen, were similar ($p > 0.05$) and not affected by replacing corn with SPP as a prebiotic in the broiler diets.

Table 3. Effects of dietary shredded steam-exploded pine particle supplementation on the organ weight (percent body weight) at the 28th day of broiler chickens.

Parameters	0%	1%	2%	pSEM	*p*-Values
Duodenum (%)	0.46	0.44	0.45	0.017	0.871
Jejunum (%)	1.04	0.97	1.01	0.026	0.584
Ileum (%)	0.95	0.87	0.84	0.029	0.270
Liver (%)	2.62	2.68	2.78	0.085	0.769
Spleen (%)	0.11	0.10	0.10	0.004	0.629

Chickens were fed with diets containing 0% (control), 1%, and 2% shredded, steam-exploded pine particles (SPPs) from the 8th day to the 28th day of age. Data show mean ($n = 8$). Abbreviations: pSEM: pooled standard error of the mean.

No variations ($p > 0.05$) were seen in the length of the intestinal organs, such as the duodenum, jejunum, and ileum, of chickens fed with an increasing concentration of SPPs in their diets (Table 4).

Table 4. Effects of dietary shredded steam-exploded pine particle supplementation on the organ length (cm) at the 28th day of broiler chickens.

Parameters	0%	1%	2%	pSEM	*p*-Values
Duodenum (cm)	27.1	27.4	26.4	0.46	0.668
Jejunum (cm)	57.6	58.3	57.0	0.84	0.845
Ileum (cm)	55.9	56.3	53.3	0.95	0.389
Cecum (cm)	15.4	15.6	15.4	0.32	0.950

Chickens were fed with diets containing 0% (control), 1%, and 2% shredded steam-exploded pine particle (SPPs) from the 8th day to the 28th day of age. Data show mean (n = 8). Abbreviations: pSEM: pooled standard error of the mean.

No significant effects ($p > 0.05$) were observed for the plasma biochemicals, such as glucose, total protein, triglyceride, and total cholesterol, among the treatment groups (Table 5).

Table 5. Effects of dietary shredded steam-exploded pine particle supplementation on the blood plasma biochemicals at the 28th day of broiler chickens.

Parameters	0%	1%	2%	pSEM	*p*-Value
Glucose (mg/dL)	260	2485	235	6.6	0.310
Total Protein (g/dL)	3.28	3.00	2.95	0.1	0.502
Triglyceride (mg/dL)	25.8	22.9	16.9	2.4	0.305
Cholesterol (mg/dL)	122	130	129	3.1	0.572

Chickens were fed with diets containing 0% (control), 1%, and 2% shredded steam-exploded pine particle (SPPs) from the 8th day to the 28th day of age. Data show mean (n = 8). Abbreviations: pSEM: pooled standard error of the mean.

A total of 24 cecum samples from three treatment groups were used for metagenomic analysis. However, three samples (one from each treatment group) were removed at the time of initial screening. After screening for quality control through chimeric reads and 97% sequence similarity, 585,545 sequences were generated that ranged between 18,561 to 41,782. From each sample, 146 to 231 OTUs were generated via clustering analysis with an abundance higher than 0.005%, resulting in a total of 4185 OTUs.

The occurrence of a sufficient number of bacterial communities in the chicken cecum samples was detected by plotting a rarefaction curve for chao1 (Figure 1). Adequate sampling depth was observed from most of the samples and was represented by flattening of the rarefaction curves towards the right, ultimately reaching a plateau.

For alpha-diversity analysis, community richness and diversity were analyzed using OTUs, Chao1, Shannon, Inverse Simpson, and Goods coverage indices (Table 6). No significant differences ($p > 0.05$) were observed in the alpha-diversity indices among the treatment groups.

Table 6. Effects of dietary shredded steam-exploded pine particle supplementation on the community richness and cecum microflora diversity at the 28th day of broiler chickens based on alpha-diversity analysis.

Parameters	0%	1%	2%	pSEM	*p*-Value
OTUs	199	203	202	4.6	0.988
Chao1	218	222	220	5.6	0.953
Shannon	4.2	4.0	4.6	0.2	0.438
Inverse Simpson	0.82	0.823	0.869	0.02	0.610
Goods coverage	0.999	0.999	0.999	0	1.0

Chickens were fed with diets containing 0% (control), 1%, and 2% shredded steam-exploded pine particle (SPPs) from the 8th day to the 28th day of age. Data show mean (n = 7). Abbreviations: OTU: operational taxonomic unit; pSEM: pooled standard error of the mean.

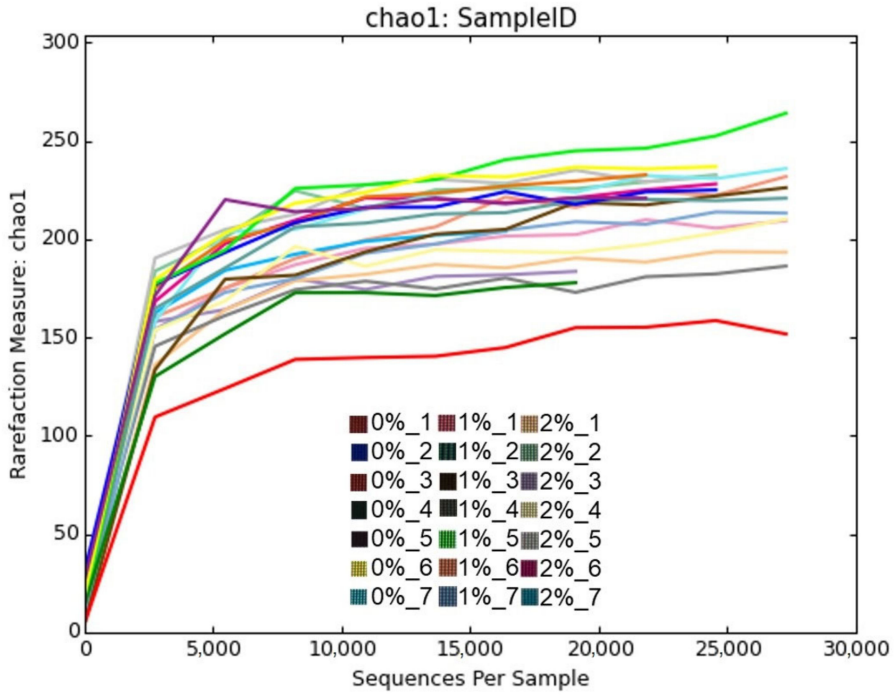

Figure 1. Rarefaction curve of Chao1 from cecum microbiota of 28th day broilers. Each line represents 21 different samples for three treatment groups. The treatments were 0% (control), 1%, and 2% steam-exploded pine particles (n = 7) in the broiler diet from the 8th day to the 28th day of age.

The PCoA based on unweighted unifrac distances were similar (Figure 2A), whereas weighted unifrac distances showed around 75% cumulative variability based on the two dimensions of PCoA, contributing 59.9% and 15.3% of the inertia, respectively (Figure 2B).

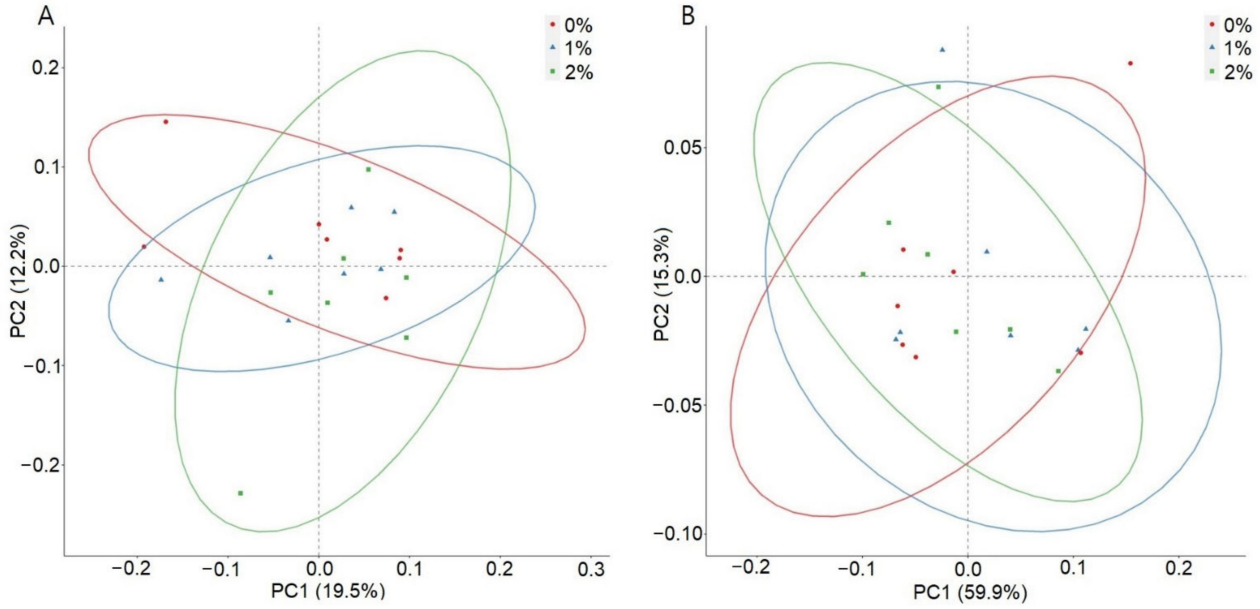

Figure 2. The principal coordinate analysis (PCoA) presenting unweighted (**A**) and weighted (**B**) unifrac distances from the cecum microbiota of 28th day broilers. The treatments were 0% (control), 1%, and 2% steam-exploded pine particles (SPPs) (n = 7) in the broiler diet from the 8th day to the 28th day of age.

To evaluate the effects of SPP supplementation on the modulation of the cecum microbial community, phylum and genus were analyzed. The bacterial community in the

phylum was similar and not significantly affected by the treatment groups. *Firmicutes* and *Bacteroidetes* were the two most abundant phyla dominating bacterial communities present in the cecum of 28-day-old broiler chickens (Figure 3).

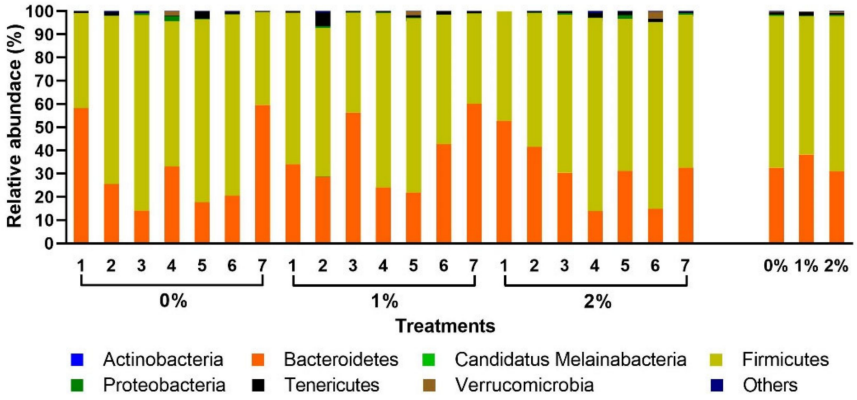

Figure 3. Effects of dietary steam-exploded pine particles (SPPs) on the composition of cecum microflora at the phylum level on the 28th day of broiler age. The treatments were 0% (control), 1%, and 2% SPP (n = 7) in the broiler diet from the 8th day to the 28th day of age.

Firmicutes and *Bacteroidetes* were found to be inversely correlated (Spearman $R = -0.981$, $p = 0.001$) with each other (Figure 4). *Bacteroidetes* were also found to be inversely correlated with the *Tenericutes* (Spearman $R = -0.378$, $p = 0.091$) and other (Spearman $R = -0.791$, $p = 0.001$) bacteria present in the phylum. However, *Actinobacteria* (Spearman $R = 0.500$, $p = 0.021$), *Firmicutes* (Spearman $R = 0.764$, $p = 0.001$), and *Tenericutes* (Spearman $R = 0.442$, $p = 0.045$) were positively correlated with the other bacteria in the phylum. *Tenericutes* was positively correlated with *Actinobacteria* (Spearman $R = 0.376$, $p = 0.093$) and *Candidatus Melainabacteria* (Spearman $R = -0.369$, $p = 0.099$).

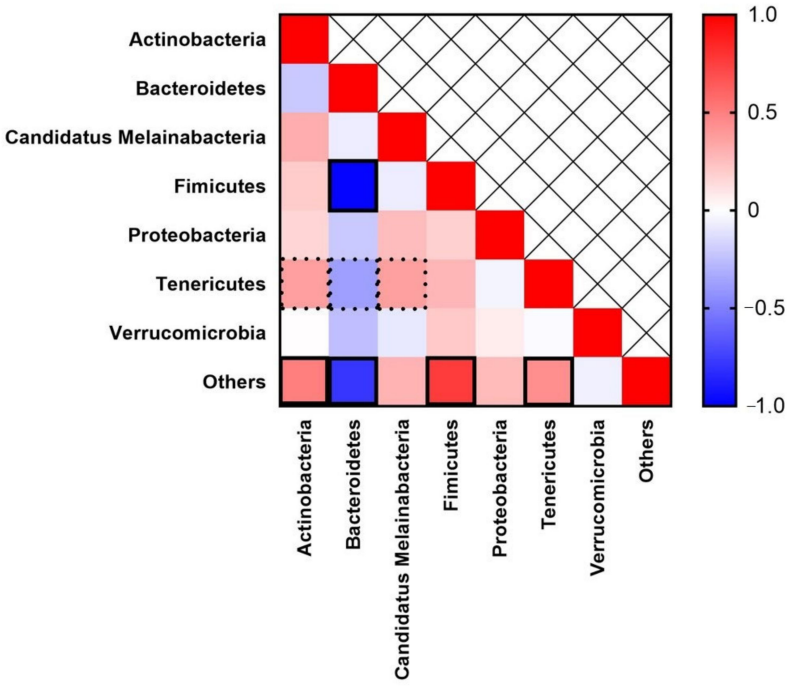

Figure 4. Correlation among the bacterial phylum of the cecum in broiler chickens. Zero indicates similarity without any possibility of correlation, while plus and minus values indicate positive and negative correlation, respectively. The continuous line and dashed line indicate significance at $p < 0.05$ and $p < 0.10$, respectively.

The effect of SPP supplementation on the cecum microbiota at the genus level is presented in Figure 5. The top three dominant genus microbiotas were *Bacteroides* (0% SPP: 25.8%, 1% SPP: 32.0%, and 2% SPP: 23.0%), *Blautia* (0% SPP: 17.5%, 1% SPP: 12.7, and 2% SPP: 7.9), and *Alistipes* (0% SPP: 6.8%, 1% SPP: 6.3, and 2% SPP: 7.9).

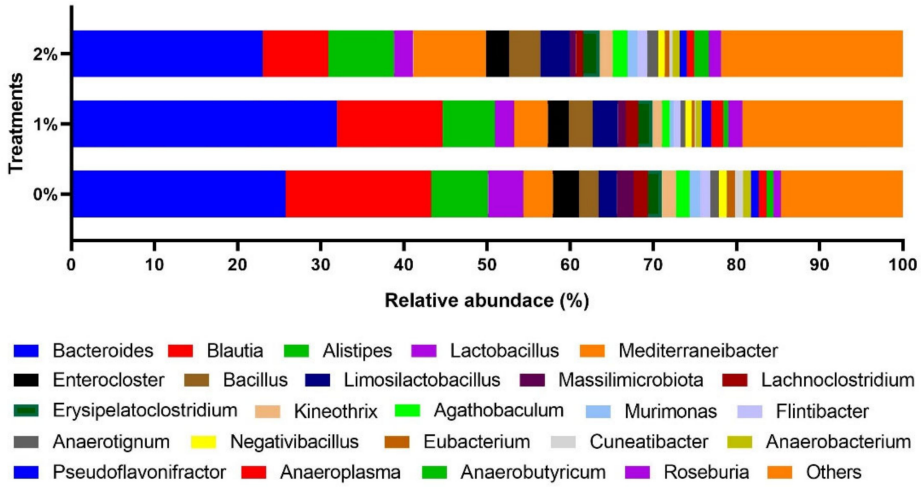

Figure 5. Effects of dietary steam-exploded pine particles (SPPs) on the composition of cecum microflora at the genus level on the 28th day of broiler age. The treatments were 0% (control), 1%, and 2% SPPs (n = 7) in the broiler diet from the 8th day to the 28th day of age.

Anaerobutyricum and *Mediterraneibacter* were the highly significant genera that were enhanced ($p < 0.05$) in 2% SPP in comparison to 1% and 0% SPP-supplemented diets in chickens (Figure 6). One more genus had a higher mean percent in 2% SPP- in comparison to 1% SPP-supplemented diets in chickens. However, the sequence of this genus is still unidentified.

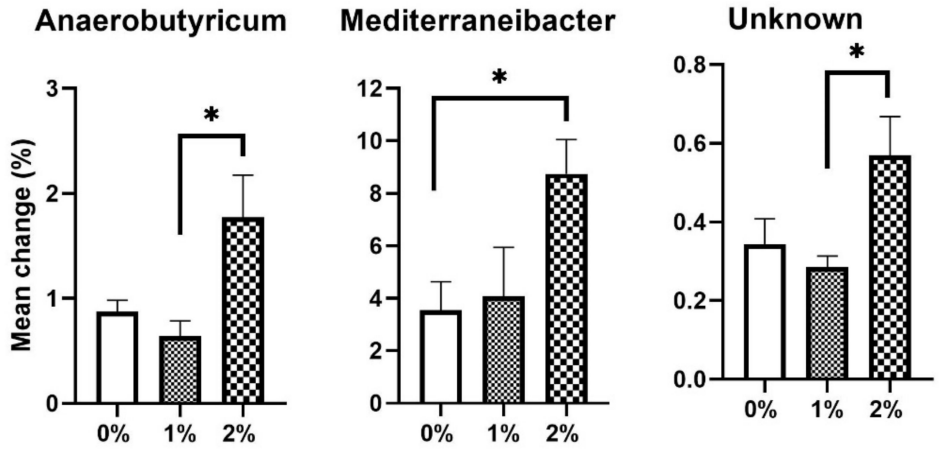

Figure 6. Effects of dietary steam-exploded pine particles (SPPs) on the significantly modified genus in the 28th day cecum of broilers. The treatments were 0% (control), 1%, and 2% SPPs (n = 7) in the broiler diet from the 8th day to the 28th day of age. * indicates a significant difference ($p < 0.05$).

4. Discussion

The SPP contains indigestible fibers and lignin that can act as a prebiotic in chicken diets. Various prebiotic products, such as mannanoligosaccharides (MOSs) and fructooligosaccharides (FOSs), have already been used in chicken diets [15,22]. In the present study, we used fibers from a pinewood source. Moreover, we used the steam explosion method to enhance the utilizable fiber. The benefit of using SPPs as a prebiotic is due to its cheap and sufficient availability.

In contrast, the availability of specialized prebiotic products, such as MOS, is inadequate and better techniques for the economical production of such products are yet to be explored to make it competitive enough to be used in livestock production [23]. Furthermore, inconsistent results have been obtained by dietary supplementation of prebiotic products on growth performances. For instance, Attia and his group reported enhanced growth performance of chickens after supplementing prebiotic products, such as MOS, in chicken diets [15]. Similarly, Mookiah and his group also reported an increase in chicken growth performances after dietary supplementation of a prebiotic product [24]. Contrary to this, other researchers reported that dietary supplementation of prebiotics, such as FOS or MOS, did not affect the growth performance in chickens [22,23]. The discrepancy in the results could be attributed to the difference in the source of prebiotic products utilizing different raw materials and microorganism enzymatic activity, resulting in differential hydrolysis of polysaccharides [23]. The motivation for using dietary fibers in the present study was different from those, such as FOS and MOS. The present study was conducted to utilize the byproducts from the wood industry while that of using specialized prebiotic products is to increase growth performances in chickens, making it incomparable for its effects on growth performance. However, an increase (42 g; $p > 0.05$) in the final body weight of 2% SPP-supplemented chickens in a 21-day feeding trial in comparison to the control indicates the potential of SPP to positively influence the body weight in chickens. Further studies are warranted to evaluate the effect of dietary SPP after long-term supplementation.

A study conducted on steam-exploded *Quercus mongolica* supplementation in chicken diets reported significantly lower ADG with increasing *Quercus mongolica* concentration in comparison to the control [6]. Contrary to this, no significant variations were seen in ADG in SPP-supplemented diets in the present study. These results could be due to the difference in the source of wood used in the studies. No negative effects on growth performance parameters indicate that supplementation of up to 2% SPP in chicken diets can consume industrial wood waste.

The gut is a vital organ responsible for the digestion and absorption of nutrients. Supplementation of dietary fibers has been known to possess modulatory roles in chickens by modifying gut health. We evaluated the relative organ weight and absolute length, which were similar and not affected by supplementation of increasing amounts of SPP in chicken diets. Our finding correlates with those of Wang et al. They reported a similar relative organ weight and absolute length at 14 and 27 days of age after dietary supplementation of an increasing concentration of prebiotics [25]. The absorptive area is dependent on the length of the intestine and is directly correlated to nutrient digestion and body weights [26]. We did not evaluate the digestion of nutrients. However, the similar ADG, ADFI, and FCR values in the current study correspond to no effects on intestinal length and weight in chickens. Furthermore, with increasing age, the absorptive capacity of the intestine depends mainly on the villus surface [27]. This indicates that if the supplementation of dietary fibers, such as SPPs, been done for a more extended period, it may have improved the gut responses in chickens.

Steam explosion is directly correlated with the destruction of vegetative structure and its severity results in increasing cellulose and hemicellulose damage [28,29]. In the present study, plasma glucose levels showed a decreasing trend ($p > 0.05$) with an increasing concentration of SPP-supplemented diets in comparison to the control. The reason behind this could be related to the availability of lower carbohydrates responsible for energy production due to the higher cellulose damage in SPP-supplemented diets. Similarly, numerically lower values ($p > 0.05$) of plasma total protein were also observed in the SPP-supplemented diets in the present study. This can be attributed to the increase in inevitable crude protein and amino acid endogenous losses in broilers fed high fiber levels [30]. Previous studies conducted on chickens fed modified dietary fiber from cassava pulp reported a decrease in fat, triglyceride, and cholesterol levels in chickens [31]. Although not significant, numerically lower plasma triglyceride was seen in 2% SPP-supplemented diets in the present study.

Dietary fibers may act as prebiotic substances that can modulate the intestinal microbiota [10]. It is expected that the non-digested fibers may act as a source for the proliferation of beneficial bacteria in the chicken gut. Recent studies suggested that dietary supplementation of alfalfa meal stimulated the proliferation of beneficial bacteria, such as *Lactobacillus* and *Bacteroides*, and inhibited potentially pathogenic bacteria including *Clostridium* [32]. Additionally, dietary supplementation of chicory forage has benefited intestinal health by increasing the microbial community diversity in the caecum of chickens [33]. Similarly, it was reported that orange and grapefruit peels, which are rich in fiber content and used as feed additives in broiler diets, significantly modulated the gut microbiota profile [34]. Contrary to our results, a study conducted by adding tannins and bacitracin to chickens' diet reported similar OTUs to that of the control, while the cecal richness of bacitracin-treated animals remained significantly lower [35]. In the present study, we used SPPs as a feed additive that was produced by pretreating pine particles (wood powder) with steam explosion to modify the fraction in terms of its fiber contents. However, this discrepancy can be attributed to the differences, such as the additives, broiler genetic line, and trial duration (i.e., 28 vs. 42 days), thereby hindering the comparison between differently designed experiments. Furthermore, the cecum plays an important role in breaking up non-digestible fibers through the process of anaerobic fermentation [11]. Thus, the evaluation of microbiota in the cecum through metagenome analysis may facilitate the determination of the beneficial effects of dietary SPPs in the chicken gut.

In general, OTUs are an indicator of relative richness, whereas Chao1 indicates the estimated richness of microflora. Similarly, Shannon, Inverse Simpson, and Goods coverage are indicators of microbial diversity. The lack of variation in the richness and diversity indicators suggests that SPP supplementation did not modify the microbial community. However, previous studies suggested that prebiotic substances as a source of fiber enhance the proliferation of favorable bacteria in the gut [10]. A recent study conducted on chickens by supplementing 4% dietary fiber from 9 weeks to 20 weeks of age had higher alpha-diversity indices than 2% fibers in diets [36]. These results confirm the role of dietary fiber in positively modifying the alpha-diversity in chickens. However, the lack of variation of the alpha-diversity indices in the present study could be due to the lower concentration (1% and 2% SPPs) of dietary SPPs and shorter period (3 weeks) of feed supplementation. Fluctuation of microbiota occurs in the early few weeks of age and can be noticed until 25 days of age [37]. The ceca in the present experiment were collected on the 28th day of age, and thus an unstable microbial population may also lead to non-significant results.

The beta diversity was evaluated by using unweighted and weighted unifrac distances. Unweighted unifrac distances are used to identify the differences in existing or missing organisms. The similarity in the PCoA of unweighted unifrac distances indicates identical species in all the treatment groups. Weighted unifrac distances are used to differentiate the abundance of microbes. Dimension one and two of PCoA from weighted unifrac contributed 59.9% and 15.3% of the inertia, respectively, making the overall variability around 75%, indicating differences in the abundance of organisms among treatment groups. Differences in the abundance of the bacteria could be attributed to the availability of fibers from the dietary source supplemented with an increasing concentration of SPPs. The possibility of differences in the abundance of organisms could also exist due to the fluctuation in the microbiota during the first four weeks of age [37], which requires further investigation with long-term experiments.

In the present study, *Firmicutes* was the most abundant phyla followed by *Bacteroidetes* and were found to be inversely correlated. These findings are in accordance with the previous studies showing that *Firmicutes* and *Bacteroidetes* were the dominant phyla throughout the ceca of chickens with the age of 7 days to 42 days [38]. However, in another study, *Bacteroidetes* was found to be the most abundant phylum in the chicken cecum [39]. The difference in the results could be related to the different farm conditions, such as temperature and hygiene. The action of the bacterial phylum takes place through SCFAs. Acetate, butyrate, and propionate are the major SCFAs in chickens, which were found to be

produced in the cecum of a high-fiber feed substrate when tested in vitro [40]. *Firmicutes* is the main producer of butyrate, while *Bacteroidetes* is mainly responsible for producing acetate and propionate in chickens [41]. This indicates that dietary fiber in SPP supplementation may help maintain the SCFAs in chickens. Heat stress has been associated with modified phylum microbiota in chicken [42]. Although we did not evaluate the effect of temperature or other aspects, modified fiber supplementation could effectively maintain the bacterial phylum in chickens and open the way for future research. In the present study, *Firmicutes* and *Tenericutes* were positively correlated. *Tenericutes* was found to have an inverse relationship with *Bacteroidetes*. Our findings agree with the previous finding that *Firmicutes* and *Tenericutes* also show a similar increasing trend in chickens [42].

Bacteroides, *Blautia*, and *Alistipes* were the three most abundant bacterial genera found in the cecum of the present study. *Blautia* is known to metabolize glucose and dietary cellulose to produce SCFAs [43,44]. However, in the present study, the abundance of *Blautia* showed a decreasing trend ($p > 0.05$). The reason behind this could be related to the increase in the cellulosic damage in the SPP-supplemented diets due to the steam-explosion method applied for its production [28,29]. The direct association can also be seen with the decrease in the plasma glucose level in this study. In the present study, *Mediterraneibacte*r and *Anaerobutyricum* are the only two known genera that also belong to the top 24 bacterial genera that were found to be significantly modified by the treatment. According to Togo et al. [45] and Shetty et al. [46], *Mediterraneibacte*r and *Anaerobutyricum* are the reclassifications of *Ruminococcus* and *Eubacterium*, respectively. Due to the presence of high fiber in diets, we expect that fiber-degrading genera could have a higher abundance in the cecum of chickens fed SPP-supplemented diets. Previous studies suggested that *Ruminococcus* and *Eubacterium* are two known fibrolytic bacterial genera that can degrade fibers through enzymatic action [47,48]. They can degrade dietary fiber, especially from plant sources [49]. The higher availability of fibers in the 2% SPP-supplemented diets may have increased the abundance of *Mediterraneibacte*r and *Anaerobutyricum* in the cecum. One more unidentified genus, with a higher abundance in 2% SPP compared to 1% SPP, was observed and needs further investigation.

5. Conclusions

In conclusion, supplementation of up to 2% of SPPs in chicken diets can be used to utilize the waste from the wood industry. Dietary supplementation of up to 2% SPP has no adverse effects on growth performance parameters in chickens. However, its systematic utilization may positively influence growth. The possibility for extended benefits of SPPs could be mediated through the bacterial genera that are responsible for degrading the fibrous contents. Conformingly, 2% dietary SPP supplementation enhanced the abundance of fibrolytic bacterial genera, such as *Mediterraneibacte*r and *Anaerobutyricum*, in the cecum, which may facilitate the breakdown of additional fibers available in the diets. Further studies are warranted to confirm the effects of dietary supplementation of SPPs in different breeds of chickens.

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