

Effect of Adding Stems and Leaves of *Scutellaria baicalensis* Georgi to Feed on the Microbial Diversity of Cattle Feces

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Abstract

Scutellaria baicalensis Georgi has important effects on anti-inflammation and anti-oxidation. However, there are few research reports on the application of *Scutellaria baicalensis* Georgi stems and leaves as the roughage in animal husbandry. Therefore, this study aims to explore the effect of adding different proportions of *Scutellaria baicalensis* Georgi stems and leaves to feed on the microbial diversity of bovine feces. Twenty Simmental cattle were randomly divided into four groups: CON, adding 3%, 6% and 9% *Scutellaria baicalensis* Georgi stems and leaves. All four groups were treated for 105 days. We need to collect fresh faecal samples on 105d and study the microbiome of cattle feces using 16S rRNA gene sequencing. The NMDS analysis showed substantial differences in bacterial community composition between the treatment group and the CON group. At the phylum level, adding *Scutellaria baicalensis* Georgi stems and leaves can significantly reduce the *Firmicutes*/*Bacteroides* ratio. After adding 3%, 6%, and 9% *Scutellaria baicalensis* Georgi stems and leaves, the relative abundance of *Spirochaetes* decreased. After adding 9% *Scutellaria baicalensis* Georgi stems and leaves, the relative abundance of *Verrucomimicrobia* and *Proteobacteria* decreased. These inter group differential microbiota mainly participates in the Amino Acid Biosynthesis, Fatty Acid and Lipid Biosynthesis, Carbohydrate Biosynthesis, Carbohydrate Degradation, Nucleoside and Nucleotide Degradation, Fermentation, Glycolysis, TCA cycle, Glycan Biosynthesis and Glycan Degradation pathway. Therefore, our research shows that adding *Scutellaria baicalensis* Georgi stems and leaves to feed can improve cattle's antioxidant capacity, reduce harmful gut bacteria and inflammatory reactions, and promote healthy growth of beef cattle. This provides a new strategy for future cattle farming.

1. Introduction

With the rapid development of the Chinese traditional herbal medicine (CTHM) industry, the scope and scale of Chinese herbal medicine cultivation have been continuously expanding. Chinese herbal medicine has a large storage capacity, low production costs, good prevention and control effects and minimal toxic side effects. It has gradually been applied as a new type of feed in practical production. There is a large amount of research foundation on Chinese herbal medicine as livestock and poultry feed. Wang et al found that adding Guizhi Li-Zhong Tang (GLZ) to piglet feed can prevent pneumonia in weaned piglets[1]. Chen et al found that adding Fermented Chinese Herbal Medicine to feed can promote growth performance, improve intestinal health and regulate bacterial flora in weaned piglets[2]. But currently, there is almost no research on adding *Scutellaria baicalensis* Georgi (SBG, an important Chinese herbal medicine in western China) as a ruminant silage feed.

Scutellaria baicalensis Georgi, a common Chinese medicinal herb that has been in use for more than 2000 years[3]. The stems and leaves of *Scutellaria baicalensis* Georgi contain nutrients such as flavonoids, saponins, sugars, amino acids and various trace elements, which have pharmacological effects such as clearing heat, drying dampness, purging fire[4], detoxifying[5], anti-inflammatory[6], antitumor[7], antioxidant[8] and immune regulation[9]. In terms of human diseases, studies have shown that Huangcen and its extract can effectively treat type II diabetes[10], lung cancer[3], autoimmune

diseases[11], ovarian cancer[12], breast cancer[13], hepatitis and colitis[14]. In addition, Wang et al found that Baicalin, the main bioactive extract of *Scutellaria baicalensis* Georgi Georgi, can protect LPS-induced Blood–brain barrier damage and activate Nrf2-mediated antioxidant stress pathway[15]. Qian et al.'s study showed that *Scutellaria baicalensis* Georgi stems and leaves can enhance mouse neurogenesis and improving memory improvement induced by the PI3K-AKT-CREB signaling pathway[16]. Sun et al found that the extract of *polysaccharide* (SPS) from *Scutellaria baicalensis* Georgi helps to improve the immunity and antioxidant capacity of giant freshwater prawn[17]. The study by Zhang et al. shows that adding *Scutellaria baicalensis* Georgi to piglet feed can alter the gut microbiota composition of piglets, which is beneficial for increasing weaning weight and promoting piglet health[18]. However, there are few studies on the addition of *Scutellaria baicalensis* Georgi stems and leaves to ruminant silage feed. So this study aims to explore the impact on the microbial diversity of cattle faeces by adding different proportions of *Scutellaria baicalensis* Georgi stems and leaves to the feed, providing a theoretical basis for adding Chinese herbal medicine formulas to cattle feed in the future.

2. Materials And Methods

2.1 Animals and Feeding Strategy

Simmental cattle (Simmental) with good body weight (385.67 ± 39.21 kg) × 20 local yellow cattle were randomly divided into 4 groups with 5 bulls in each group. The experimental diet was prepared according to the NY/T 815-2004 standard. The control group did not add *Scutellaria baicalensis* Georgi stems and leaves (accidental death of one cow in the CON group during the experiment), while the experimental group added 3%, 6% and 9% *Scutellaria baicalensis* Georgi stems and leaves, respectively. The specific dietary composition and nutritional levels are shown in Table 1. At the same time, each cow is supplemented with 1 kg of a certain commodity beef concentrate supplement pellet feed every day, and its nutritional composition is shown in Table 2.

2.2 Sample Collection and Processing

The process of collecting and processing samples includes:(1) Collect faecal samples from cattle after 105 days of treatment. (2) Fix the cattle and collect fresh sterile faecal samples when they defecate. (3) Immediately store them in sterile tubes and quickly freeze them in liquid nitrogen, and then store them at -80°C for microbial diversity analysis.

2.3 16S rRNA Gene Sequencing

The total genomic DNA of the sample was extracted through the MP Biomedicals Kit (America, MP Biomedicals), and the DNA concentration and purity were detected on 1% agarose gel. The concentration of each DNA sample was diluted to 1ng/μL with sterile water. Then, 16S rRNA genes in different regions were amplified using specific primers with a bar code (515F/806R). Then, 15μL of Phusion was added to

the PCR reaction for high-fidelity PCR with the main mixture (New England biological laboratory) using 2 μ M forward and reverse primers and approximately 10 ng of the template DNA. The thermal cycle included an initial denaturation at 98°C for 1 min, denaturation at 98°C for 30 times for 10 s, annealing at 50°C for 30 s, extension at 72°C for 30 s, and a final holding at 72°C for 5 min. The same volume of 1X loaded buffer (including the SYBR Green) was mixed with the PCR product and detected by 2% agarose gel electrophoresis.

The PCR products were mixed in an equal density ratio. Then, the Qiagen Gel Extraction Kit (German Qiagen) was used to purify the mixed PCR products. TruSeq was recommended by the manufacturer of the DNA free PCR sample preparation kit (Illumina, United States) to generate the sequencing library and add the index code. The quality of the library was evaluated through Qubit@ 2.0 Fluorometer (Thermo Scientific) and the Agilent Biological analyzer 2,100 system. Finally, the library was sequenced on the Illumina NovaSeq platform, and 250-bp paired-end read codes were generated.

2.4 Metagenomic Data Analyses

According to the bar code and PCR amplified primer sequences, each sample data was separated from the offline data. The bar code and primer sequences were intercepted with the FLASH (V1.2.7, <http://ccb.jhu.edu/software/FLASH/>). The reads of each sample were spliced, and the spliced sequences were the original data tags (raw tags). We referred to the Qiime (V1.9.1, http://qiime.org/scripts/split_libraries_fastq.html) process to obtain a high-quality tag sequence. These tags sequences were passed (<https://github.com/torognes/vsearch/>). The species' annotation databases were compared to detect the chimera sequence. The chimera sequences were finally removed to obtain the final adequate data.

Using the uparse algorithm (uparse v7.0.1001, <http://www.drive5.com/uparse/>), all the effective tags of samples were clustered. By default, the sequences were clustered into the operational taxonomic units (OTUs) with 97% identity. Meanwhile, the sequence with the highest frequency in OTUs was selected as the representative sequence of OTUs. The music (version 3.8.31, <http://www.drive5.com/muscle/>) software was employed to perform fast multi-sequence alignment to determine the phylogenetic relationship of OTU representative sequences. Finally, the data of each sample were homogenized.

2.5 Statistical analyses

SPSS 25.0 software (IBM, Chicago, Illinois, United States) was employed for statistical analyses. The differences between the two groups were compared through a t-test. For the similarity analysis of multivariate data (Anosim), we adopted the R software (<http://www.r-project.org/>) for comparing the bacterial community structure. $P < 0.05$ was considered statistically significant.

3. Results

3.1 Species diversity analysis

Fresh faecal samples were obtained from the cattle of CON group and 3 treatment groups after 105 days of the treatment to investigate the effect of adding 3%, 6%, 9% *Scutellaria baicalensis* Georgi stems and leaves on cattle intestinal microbiota composition. A total of 113,457 labels were matched with 19 samples, with effective-quality control rate of 89.30% and high-quality control rate of 85.96% (Table 3). The rarefaction curve (average curve for each group) indicates that most microbial diversity has been fully recorded (Figure 1A). According to the Wilcoxon rank-sum test, no significant differences were observed in Chao, Observed species, Shannon, Simpson, Faith, Pielou or Goods coverage (Figure 1B) between the treatment group and the CON group ($P > 0.05$). However, some substantial differences in 3 treatment groups and CON group were observed due to adding *Scutellaria baicalensis* Georgi stems and leaves. The NMDS ranking diagram, based on Bray Curtis distance measurement, demonstrated that the faecal bacterial population in the sample was separated by adding *Scutellaria baicalensis* Georgi stems and leaves (Figure 1C). According to Anosim analysis, the differences between groups are shown in Table 4. There was a significant difference between the CON group and the group with adding 9% *Scutellaria baicalensis* Georgi stems and leaves ($P < 0.05$), as well as the group with adding 3% *Scutellaria baicalensis* Georgi stems and leaves and the 9% group ($P < 0.05$).

3.2 Differential microbiota expression

Analyzing the classification components with relative abundance greater than 1% can determine the bacterial population induced by adding *Scutellaria baicalensis* stems and leaves (Figure 2A). *Firmicutes* were the most abundant in CON group, with an average abundance of 49.47%, followed by *Bacteroidota* (32.81%). In the group of adding 3% *Scutellaria baicalensis* Georgi stems and leaves, *Firmicutes* (50.85%) also occupied an absolute advantage, followed by *Bacteroidota* (31.47%), *Spirochaetes* (8.57%), *Tenericutes* (4.08%), *Verrucomicrobia* (1.88%) and *Proteobacteria* (1.39%). In the group of adding 6% *Scutellaria baicalensis* Georgi stems and leaves, *Firmicutes* (46.62%) also occupied an absolute advantage, followed by *Bacteroidota* (35.57%), *Spirochaetes* (8.09%), *Tenericutes* (4.92%), *Verrucomicrobia* (1.43%) and *Proteobacteria* (1.23%). In the group of adding 9% *Scutellaria baicalensis* Georgi stems and leaves, *Firmicutes* (48.06%) also occupied an absolute advantage, followed by *Bacteroidota* (35.67%), *Spirochaetes* (8.90%), *Tenericutes* (4.76%), *Verrucomicrobia* (0.62%) and *Proteobacteria* (0.84%). To investigate the evolutionary relationships of species at the genus level, sample sequences from the Top 100 genera were acquired through multiple sequence alignment, as shown in Figure 2B. 20% can be annotated to the genus level. *Treponema* was the most abundant in CON group, with an average abundance of 9.19%, followed by 5-7N15 (4.13%). In the group of adding 3% *Scutellaria baicalensis* Georgi stems and leaves, *Treponema* (8.42%) also occupied an absolute advantage, followed by 5-7N15 (3.46%), *Clostridiaceae_Clostridium* (2.31%) and CF231 (1.9%). In the group of adding 6% *Scutellaria baicalensis* Georgi stems and leaves, *Treponema* (7.59%) also occupied an absolute advantage, followed by 5-7N15 (4.62%), *Clostridiaceae_Clostridium* (1.30%) and CF231 (1.58%). In the group of adding 9% *Scutellaria baicalensis* Georgi stems and leaves, *Treponema* (8.36%) also occupied

an absolute advantage, followed by *5-7N15* (4.34%), *Clostridiaceae_Clostridium* (1.82%) and *CF231* (1.62%). In addition, we have mapped the taxonomic hierarchy (Figure 2C) and evolutionary tree diagrams (Figure 2D) for the phylum, class, order, family, and genus with the highest abundance of 50 OTUs.

Wayne diagrams and species composition heatmaps were drawn to further compare the differences in species composition between samples. The Wayne diagram is shown in Figure 3A, with 1111 identical OTUs in the four groups. There are 101, 146, 138, and 121 OTUs in CON group, and adding 3%, 6%, and 9% *Scutellaria baicalensis* Georgi stems and leaves groups, respectively. Draw a heat map of the top 20 OTUs with average abundance as shown in Figure 3B. Description of OTU_131, OTU_56, OTU_808, OTU_2509, OTU_1424, OTU_83, OTU_93, OTU_2944, OTU_1792, OTU_776, OTU_2839, OTU_166, OTU_1178, OTU_167, OTU_814, OTU_2325, OTU_269, OTU_262, OTU_162 and OTU_957 is an important indicator of inter group differences.

3.3 Enrichment of differential microbiota pathways

These bacteria were subjected to a KEGG, MetaCyc and COG analysis, which revealed substantial differences (Figure 4A). Differential microorganisms were found to be mainly involved in the Nucleoside and Nucleotide Biosynthesis, Amino Acid Biosynthesis, Cofactor, Prosthetic Group, Electron Carrier, and Vitamin Biosynthesis, Fatty Acid and Lipid Biosynthesis, Carbohydrate Biosynthesis, Carbohydrate Degradation, Carboxylate Degradation, Nucleoside and Nucleotide Degradation, C1 Compound Utilization and Assimilation, Polymeric Compound Degradation, Fermentation, Glycolysis, TCA cycle, Glycan Biosynthesis and Glycan Degradation pathway through pathway enrichment. In addition, the PIZI graph shows that the five most abundant phylum species are *Firmicutes*, *Bacteroidetes*, *Spirochetes*, *Tenericulantes* and *Verrucomimicrobia* (Figure 4B).

4. Discussion

We found that *Scutellaria baicalensis* Georgi and its extracts can significantly affect the structure and diversity of Gut microbiota of animals. Wang et al found that Georgi of *Scutellaria baicalensis* Georgi can regulate the structure and diversity of Gut microbiota in rats with spleen deficiency and damp heat, and balance the metabolism of the body[19]. Fan et al.'s study showed that supplementing plant extracts of *Scutellaria baicalensis* Georgi can regulate oxidative stress and gut microbiota to alleviate colitis in mice[20]. However, as an important ruminant, beef cattle are susceptible to environmental influences that can cause inflammation and oxidative stress in the body[21]. Therefore, adding pure natural Chinese herbal medicine *Scutellaria baicalensis* Georgi may help with the health management of beef cattle. This study added 3%, 6% and 9% *Scutellaria baicalensis* Georgi stems and leaves to feed in order to explore the effect of *Scutellaria baicalensis* Georgi additives on the microbial diversity spectrum of cattle faeces.

We investigated the effect of adding *Scutellaria baicalensis* Georgi on the microbial diversity profile of cattle faeces through metagenomic sequencing. The research results show that *Firmicutes* and

Bacteroidota are the two most common phylum in cattle, which is consistent with the earlier research[22-24]. We also found changes in classification and function between the CON group and the group adding *Scutellaria baicalensis* Georgi stems and leaves in the faecal microbiome. At the phylum level, our research results show that *Firmicutes* and *Bacteroides* are the two main groups, followed by *Spirochaetes*, *Tenericutes*, *Verrucomimicrobia* and *Proteobacteria*. This is consistent with the research results of Liu et al[25] and Wen et al[23]. It is worth noting that the addition of 6% and 9% *Scutellaria baicalensis* Georgi stems and leaves significantly reduced the ratio of *Firmicutes/ Bacteroides*. Traditionally, the *Firmicutes/ Bacteroides* ratio has been associated with susceptibility to disease status[26]. Bin's study showed an increase in the proportion of *Firmicutes/ Bacteroides* in the jejunum of diarrheal piglets, and determined that the increase in *Firmicutes/ Bacteroides* ratio is related to diarrhea[27]. *Firmicutes/ Bacteroides* ratio has often been used as a marker of obesity in previous studies[28]. *Firmicutes* are associated with obesity because they can ferment plant polysaccharides to produce Short chain fatty acid (SCFA) to provide additional energy[26]. *Bacteroides* is an anaerobic Gram negative bacterium found in the gut microbiota of mammals[29, 30]. *Bacteroidetes* are crucial for plant polysaccharide decomposition and amino acid fermentation[31, 32]. This indicates that a decrease in the ratio of *Firmicutes/ Bacteroides* may weaken the adhesion and colonization of pathogens in the intestine. Therefore, we believe that adding 3% or 9% *Scutellaria baicalensis* Georgi stems and leaves to feed can help improve cattle's disease resistance and immunity, reduce diarrhea and promote healthy growth. In addition, the research of Hua et al[33] shows that the usage of Baitouweng Tang (BTWT) to treat Ulcerative colitis (UC) in mice has found that BTWT has increased the relative endurance of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Tenericutes*. The study of Nagu et al[34] suggests that *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Teneriicutes* and *Verrucomimicrobia* are involved in regulating neuroinflammation and other inflammatory processes. Therefore, we believe that adding the stems and leaves of *Scutellaria baicalensis* Georgi to the feed will help reduce inflammatory reaction, increase the diversity of Gut microbiota.

According to the functional prediction results, the inter group differential microbiota may participate in the Amino Acid Biosynthesis, Fatty Acid and Lipid Biosynthesis, Carbohydrate Biosynthesis, Carbohydrate Degradation, Nucleoside and Nucleotide Degradation, Fermentation, Glycolysis, TCA cycle, Glycan Biosynthesis and Glycan Degradation pathway. Amino acid metabolism is the central content of protein catabolism. The research of Song et al[35] shows that the changes of related amino acid metabolism can improve Hyperuricemia and inflammation in mice. The study of He et al[36] also showed that amino acids can improve the function of the intestinal barrier and the expression of anti-inflammatory cytokines, thereby reducing oxidative stress, intestinal cell apoptosis, and the expression of pro-inflammatory cytokines in intestinal inflammation. Amino acid metabolism plays an important role in preventing and treating intestinal inflammation. Strengthening the amino acid metabolism pathway may help alleviate the inflammatory response in cattle. The TCA cycle is closely related to amino acid metabolism, glucose metabolism, and fat metabolism. This is consistent with the findings in this study that the inter group differential microbiota is involved in Fatty Acid and Lipid Biosynthesis, Carbohydrate Biosynthesis,

Glycolysis, and TCA cycle. Lipids can regulate various cellular responses, including cell growth and death, as well as inflammation, through receptor mediated pathways[37]. Macrophages mainly rely on aerobic Glycolysis and fatty acid biosynthesis to maintain inflammatory response and kill pathogens. Glycolysis is a rapid method for ATP production[38], and fatty acids are precursors for the synthesis of inflammatory mediators[39]. On the other hand, macrophages can metabolize fatty acid oxidation and oxidative phosphorylation to mediate the regression of inflammation[37]. The TCA cycle is involved in the activation process of inflammatory macrophages[40]. In addition, Kelly et al. also showed that the TCA cycle intermediate succinate promotes the expression of inflammatory genes[41]. In conclusion, our results suggest that the addition of *Scutellaria baicalensis* Georgi stems and leaves may change the intestinal flora structure by regulating amino acid metabolism, carbohydrate and lipid synthesis, Glycolysis and TCA cycle, thereby reducing the inflammatory response of cattle, and adjusting the oxidative stress state, and promoting the healthy growth of cattle.

5. Conclusion

In summary, current research has revealed changes in the gut microbiota structure of cattle with the addition of different proportions of *Scutellaria baicalensis* Georgi stems and leaves. The structural changes of inter group differences in microbiota are closely related to intestinal dysfunction and oxidative stress levels. In our study, we found that the microbial communities that changed after the addition of *Scutellaria baicalensis* Georgi stems and leaves, which may have potential and beneficial characteristics. In the future, the healthy breeding of cattle can be promoted by adding *Scutellaria baicalensis* Georgi stems and leaves to the feed. This provides a strong basis for us to use Chinese herbal medicine to feed cattle in the future.

Declarations

Acknowledgement

All authors read and approved the final manuscript.

Data Availability

All the figures and tables used to support the findings of the current study are included in the article.

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Ethical approval

All applicable international, national, and institutional guidelines for the care and use of animals were followed.

Conflict of interest

No conflicts of interest.

Author Contribution

All authors contributed to the study conception and design. JL Z, Y M and XQ W designed the experiment. JL Z, X B and RN N collects samples and completes the manuscript. XJ L provided financial support. All authors read and approved the final manuscript.

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Tables

Table 1 Composition and nutrient levels of total mixed ration

Items	Control group	<i>Scutellaria baicalensis</i> stems and leaves addition group/%		
		3	6	9
Ingredient				
Straw	6.00	5.00	5.00	4.00
Reed grass	7.00	5.00	3.00	3.00
Corn cob	15.00	15.00	15.00	15.00
Corn silage	24.00	24.00	24.00	24.00
Wolfberry leaves and rake	1.20	1.20	1.20	1.20
<i>Scutellaria baicalensis</i> stems and leaves	0.00	3.00	6.00	9.00
Wheat straw	6.00	6.00	5.00	3.00
Corn	27.00	27.00	27.00	27.00
Flax meal	6.00	6.00	6.00	6.00
Wheat bran	6.00	6.00	6.00	6.00
NaHCO3	0.50	0.50	0.50	0.50
NaCl	0.40	0.40	0.40	0.40
Premix ¹	0.90	0.90	0.90	0.90
Total	100.00	100.00	100.00	100.00
Nutrient levels				
CF	18.6	19.34	20.08	20.82
CP	7.42	7.43	7.43	7.44
Ca	0.48	0.47	0.46	0.45
P	0.30	0.29	0.29	0.28
NE _{mf} (MJ/kg)	5.81	5.75	5.70	5.64

1 The premix provided following per kg of finished product: VA 40 000 ~330 000IU VD₃ 10 000 ~100 000 IU VE 600 000 IU Fe 60 ~1 600 mg Zn 60 ~400 mg Cu10 ~500 mg Mg 40 ~500mg

2 NE_{mf} was a calculated value while the other nutrient levels were measured values. The same as below.

Table 2 Nutrient level of beef concentrate supplementary pellet feed

CP %	NDF %	ADF %	EE %	Ca %	P %	NE _{mf} /(MJ/kg)
18.52	8.51	23.22	5.36	0.93	0.56	6.74

Table 3 Data preprocessing, statistics and quality control.

group	SampleID	Input	Raw PE	Effective %	High quality sequence	Rate %
CON	CK1	5,787	4,985	86.14%	4,740	81.91%
	CK2	5,138	4,496	87.50%	4,262	82.95%
	CK3	4,555	4,345	95.39%	4,295	94.29%
	CK5	5,665	4,869	85.95%	4,637	81.85%
G1	S1	5,683	4,828	84.96%	4,584	80.66%
	S2	4,858	4,563	93.93%	4,467	91.95%
	S3	7,721	6,834	88.51%	6,581	85.24%
	S4	6,321	5,547	87.76%	5,269	83.36%
	S5	5,476	5,266	96.17%	5,192	94.81%
G2	T1	6,677	5,890	88.21%	5,601	83.88%
	T2	6,127	5,426	88.56%	5,239	85.51%
	T3	5,496	5,163	93.94%	5,074	92.32%
	T4	5,869	5,114	87.14%	4,830	82.30%
	T5	5,210	4,628	88.83%	4,453	85.47%
G3	Y1	6,032	5,466	90.62%	5,289	87.68%
	Y2	6,031	5,395	89.45%	5,184	85.96%
	Y3	9,020	7,760	86.03%	7,495	83.09%
	Y4	5,209	4,965	95.32%	4,855	93.20%
	Y5	6,582	5,779	87.80%	5,476	83.20%
Total		113,457	101,319	89.30%	97,523	85.96%

Table 4 Difference between groups

Group1	Group2	Sample size	<i>P</i> -value
CON	G1	9	0.279
CON	G2	9	0.067
CON	G3	9	0.018 0.05
G1	G2	10	0.12
G1	G3	10	0.016 0.05
G2	G3	10	0.588

Figures

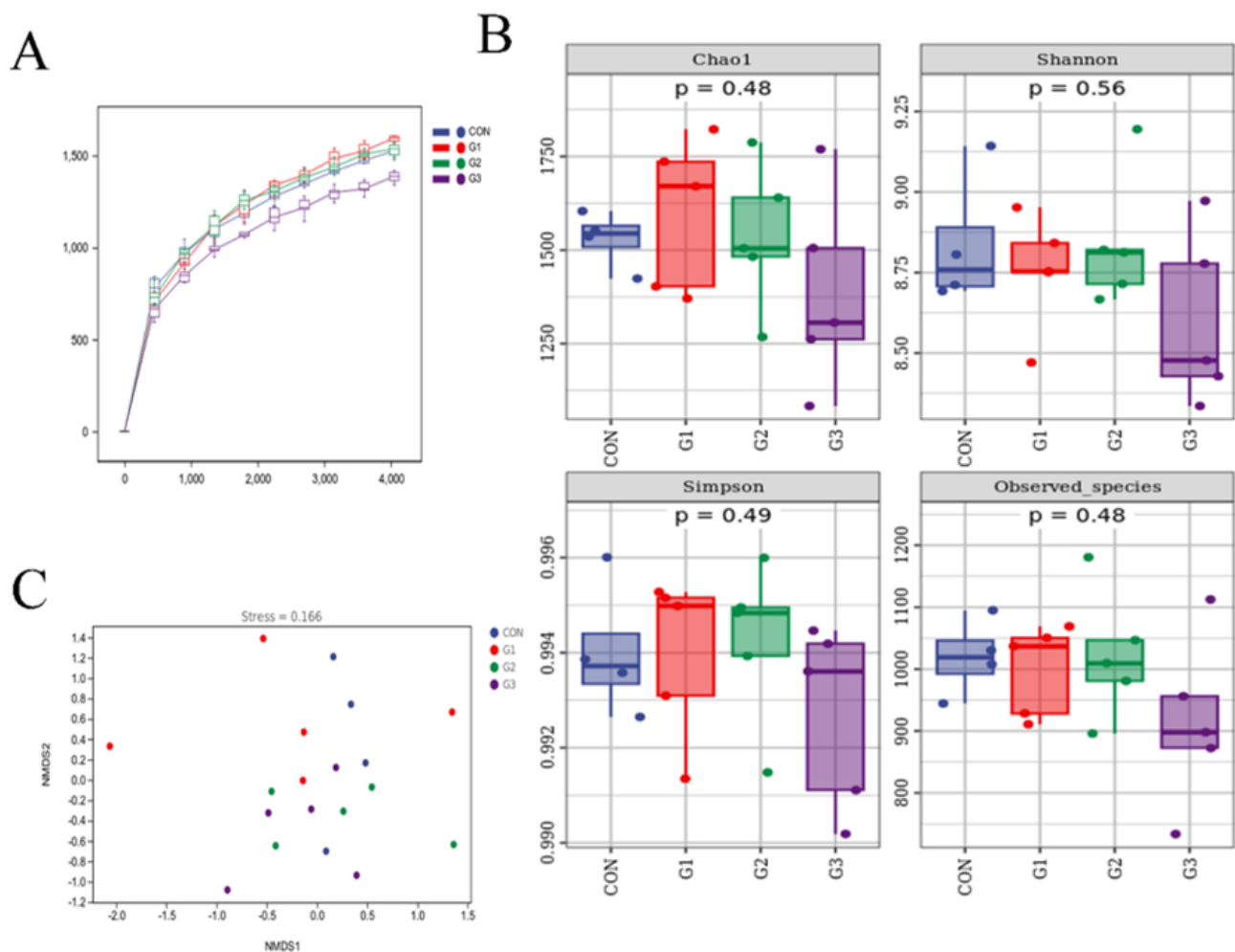


Figure 1

Species diversity analysis. (A). Rarefaction curves (the mean curves for the samples/group) were used to plot the number of phylotypes detected in the 16S rDNA gene libraries by the number of sequences from the faecal microbiota of cattle in the CON, G1, G2 and G3 groups; (B). Box chart of Chao, Shannon, Simpson and Observed_species index; (C). Non-Metric Multi-Dimensional Scaling (NMDS) ordination plots of the faecal bacterial communities in the CON, G1, G2 and G3 group based on the Bray–Curtis distance metric. CON group = did not add *Scutellaria baicalensis* Georgi stems and leaves; G1 group = added 3% *Scutellaria baicalensis* Georgi stems and leaves; G2 group = added 6% *Scutellaria baicalensis* Georgi stems and leaves; G3 group = added 9% *Scutellaria baicalensis* Georgi stems and leaves; the same below.

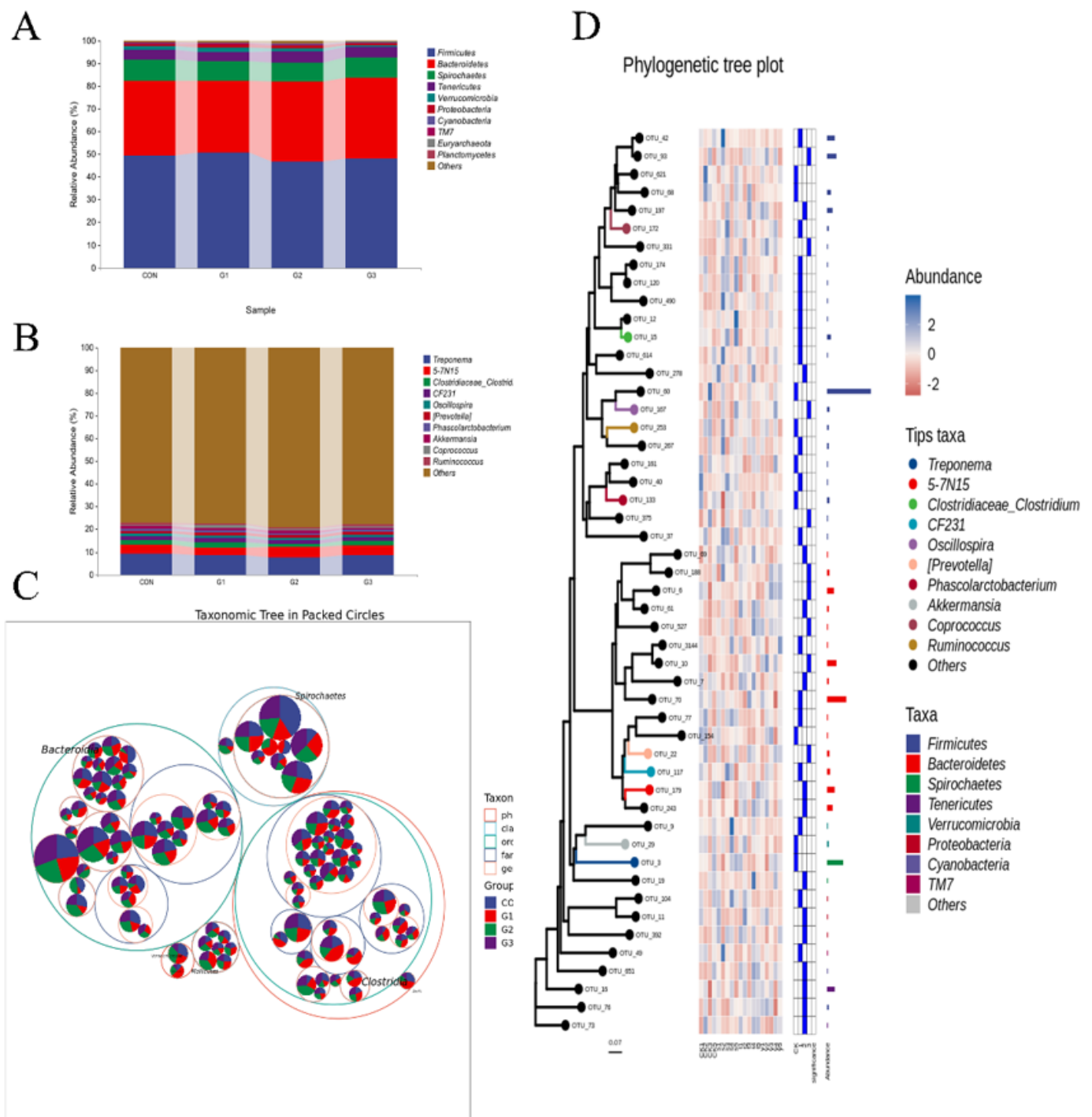


Figure 2

Effect of adding different ratios of stems and leaves of *Scutellaria baicalensis* Georgi on faecal microbial diversity. (A). Histogram of species relative abundance at the phylum level; (B). Histogram of the species' relative abundance at the genus level; (C). Phylum level taxonomic hierarchy; (D). Evolutionary tree diagrams for the phylum, class, order, family, and genus with the highest abundance of 50 OTUs.

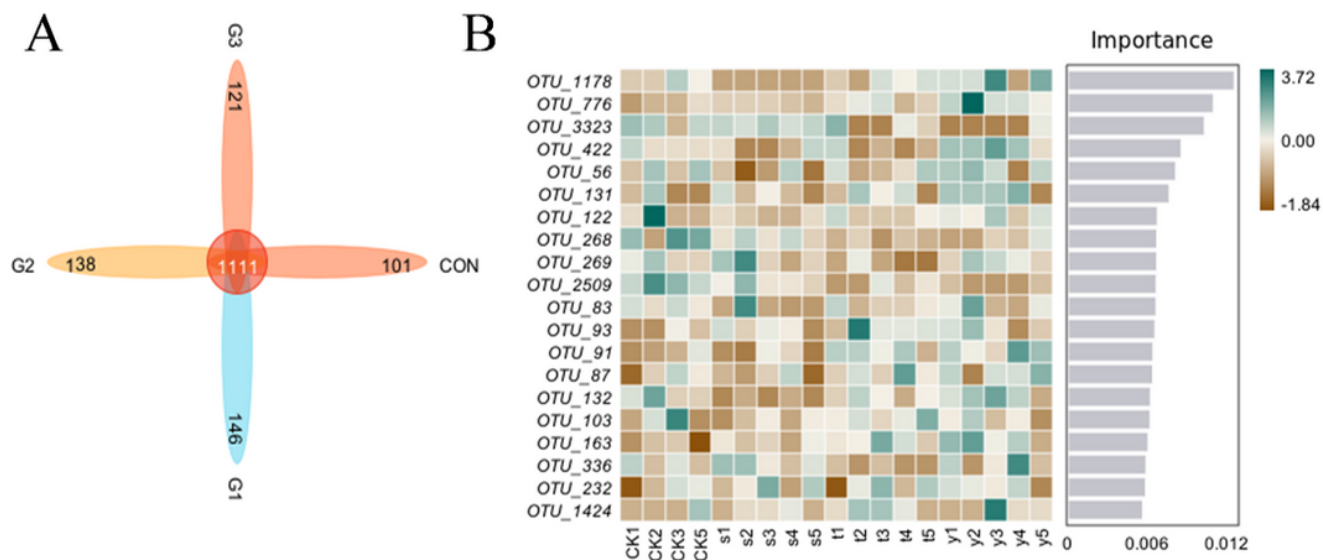


Figure 3

Effect of adding different ratios of stems and leaves of *Scutellaria baicalensis* Georgi on faecal microbial diversity. (A) Wayne diagrams; (B) Heat map of the top 20 OTUs.

A



B

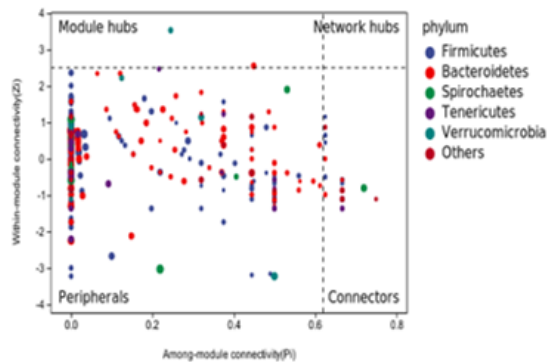


Figure 4

Enrichment of differential microbiota pathways. (A). KEGG, MetaCyc, and COG Pathways Enriched by Intergroup Differences in Microbial Communities; (B). PIZI graph.