

Effects of Compound Probiotics on Growth Performance and Intestinal Flora of Kunming Mice

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Abstract [**Objectives**] This study was conducted to investigate the effects of adding compound probiotics on the growth performance and intestinal flora of Kunming mice. [**Methods**] Twelve healthy 2-week-old Kunming male mice with body weight of (11.09 ± 0.43) g were selected. They were randomly divided into two treatment groups, namely blank control group (NC) and compound probiotics group (CB + LR + BS), with six mice in each group. The two groups were fed with commercial basal diet, and the compound probiotic experimental group was fed with basal diet supplemented with compound probiotics, in which the contents of *Clostridium butyricum* spores, *Lactobacillus reuteri* and *Bacillus subtilis* spores were 1×10^{10} , 1×10^{11} and 1×10^{10} CUF/kg, respectively. The body weight, feed intake and water intake of mice were counted every 4 d, and the experimental period was 13 d. On the 13th day, the cecal contents of the mice were collected for analysis. [**Results**] There was no significant change in body weight and feed intake when compound probiotics were added to the diet. However, the addition of compound probiotics reduced the abundance of harmful bacteria such as *Escherichia coli*, urease-negative *Helicobacter typhlonius* and *Salmonella enterica*, while increasing the abundance of beneficial bacteria such as *Anaerostipes hadrus*, and the contents of IgG and IgM increased significantly ($P < 0.05$). [**Conclusions**] In summary, the addition of compound probiotics could significantly improve the structure of intestinal microbial flora, increase the quantity of beneficial bacteria, reduce the quantity of harmful bacteria, and improve the immune function of mice.

Key words Compound probiotics; Third-generation sequencing analysis; Growth performance; Intestinal flora

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Since January 1, 2020, China has implemented the strictest policies of limiting, banning, and eliminating antibiotics. Probiotics have become a new type of hotspot feed additive in China's comprehensive maintenance of animal source food safety and public health safety after animal husbandry entered the antibiotic-free era.

Probiotics are living microorganisms that, when consumed in sufficient quantities, will bring health benefits to the host^[1]. Common probiotics include *Bacillus subtilis*, *Bifidobacterium*, lactic acid bacteria, yeast and so on. Probiotics are not only involved in a series of physiological reactions in health maintenance and clinical treatment, but also microbial regulators with functions of resisting inflammation and diarrhea and preventing cardiovascular and cerebrovascular diseases^[2]. More and more studies have found that the imbalance of flora exists in many diseases, such as obesity, type 2 diabetes and inflammatory bowel disease, and the balance of flora can be adjusted by adding additional beneficial bacteria to restore and maintain health^[3].

The effects of *Clostridium butyricum* and *B. subtilis* on growth performance, antioxidation and intestinal tract of livestock and poultry have been studied more. Compound feed can improve the production performance and egg quality of laying hens, enhance the immune function of the body^[4] and improve the intestinal microstructure of ducks^[5]. However, the synergistic mechanism of

C. butyricum spore-*Lactobacillus reuteri*-*B. subtilis* compound probiotics has not been studied yet. This study aimed to explore the effects of compound probiotics on the growth performance and intestinal flora of Kunming mice by adding *B. subtilis*, *C. butyricum* spores and *L. reuteri* to the basal feed for experimental mice, so as to provide new data for the strategy of probiotics promoting healthy development.

Overview of *C. butyricum*, *L. reuteri* and *B. subtilis*

C. butyricum belongs to the genus *Clostridium* and is a strict anaerobic bacterium, which is widely used in livestock production, food processing, medicine and other fields^[6]. *C. butyricum* has a good effect on human and animal health care and prevention and treatment of enteritis and other diseases, and also outstanding functions in creation of intestinal microecological balance and antioxidation. Studies have shown that *C. butyricum* can promote the repair of intestinal epithelial structural barrier in chickens, which can provide theoretical basis for the prevention and treatment of necrotic enteritis in chickens^[7]. *C. butyricum* and its metabolites can regulate the deposition of intramuscular fat in animals, thus affecting the muscle quality of livestock and poultry^[8].

B. subtilis, a Gram-positive bacterium belonging to the genus *Bacillus*, is widely used and has broad market prospects. *B. subtilis* will produce bacteriocin in the growth process, which has obvious inhibitory effect on bacteria^[9], and it can also synthesize α -amylase, lipase, cellulase and other enzymes, which play a role together with digestive enzymes in animals in the digestive tract^[10]. The fermentation products of *B. subtilis* can also inhibit a variety of pathogenic bacteria, and have the functions of preservation and

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antiseptics^[11] and improving the growth performance and humoral immunity of livestock and poultry^[12].

L. reuteri belongs to *Lactobacillus* of the phylum Firmicutes. It is a Gram-positive, non-motile and non-spore-producing special heterotypic fermentation bacteria. *L. reuteri* has strong adhesion to intestinal mucosa, which can improve the distribution of intestinal flora, antagonize the colonization of harmful bacteria and avoid intestinal diseases^[13]. *L. reuteri* can produce a non-protein broad-spectrum antibacterial substance, reuterin, which has a good antibacterial effect^[14] and even inhibits colorectal cancer^[15].

Materials and Methods

Experimental materials

The compound probiotics in the diet mainly included *C. butyricum* spores, *L. reuteri* and *B. subtilis* spores, and the contents of the three probiotics were 1×10^{10} , 1×10^{11} and 1×10^{10} CFU/kg respectively. The compound probiotics used in the experiment were provided by Hunan Xiangnong Animal Pharmaceutical Co., Ltd.

Experimental design and basal diet

Twelve 2-week-old healthy Kunming mice were divided into two treatment groups according to the principle of consistent weight. The control group was fed with a basal diet, and the experimental group was fed with a basal diet added with compound probiotics, which included *C. butyricum* spores, *L. reuteri* and *B. subtilis* spores with the contents of 1×10^{10} , 1×10^{11} and 1×10^{10} CFU/kg, respectively. The experimental period was 13 d. The animal rearing environment is a 12 h day and night alternation, with an indoor temperature around 27 °C, and all animals were free to eat and drink. The research protocol was approved by the Animal Management Ethics Committee of Hunan Xiangnong Animal Pharmaceutical Co., Ltd. (approval number: IACUC HAPC 2021-121-03), and all animals were treated in strict accordance with animal ethics procedures and norms. The composition and nutritional level of basal diet are shown in Table 1.

Table 1 Composition and nutritional level of basal diet (air-dried basis)

Ingredients	Content//%	Nutrient levels	Content//%
Corn	27	ME/(MJ/kg) ⁽²⁾	11.7
Wheat bran	19	CP	19.9
Rice	16	EE	4.0
Soybean meal	16	CF	3.6
Fish meal	13	Ash	5.5
Bone dust	3	Ca	0.94
NaCl	0.5	TP	0.73
Limestone	3		
Yeast powder	2.3		
Premix ⁽¹⁾	0.2		
Total	100		

(1) Premix in each kilogram of diet provided: VA 8 000 IU, VB₁ 6 mg, VB₂ 8 mg, VB₆ 4 mg, VD 800 IU, VE 50 IU, VE 60 IU, Cu (as copper sulfate) 10 mg, Fe (as ferrous sulfate) 80 mg, Mn (as manganese sulfate) 80 mg, Zn (as zinc sulfate) 40 mg, I (as potassium iodide) 0.40 mg, and Se (as sodium selenite) 0.10 mg. (2) Metabolic energy is calculated and the rest is measured.

Production performance measurement

From the beginning of the experiment, the weight, feed intake and water consumption of mice were recorded every 4 d, and the growth performance indexes such as average daily gain, average daily feed intake and feed-to-weight ratio were determined.

Determination of immune indexes

After the experimental mice were fasted for 12 h, they were anesthetized with pentobarbital sodium on the 13th day. Blood was drawn from their abdominal aorta, left standing for 30 min, centrifuged at 4 °C and 3 000 r/min for 15 min, and serum was separated and stored. The contents of IgM and IgG in serum were determined by enzyme-linked immunosorbent assay (ELISA) using immunoglobulin M (IgM) ELISA kit and immunoglobulin G (IgG) ELISA kit purchased from Shenzhen Mindray according to the instructions.

Determination of intestinal flora samples

During the feeding period, after fasting for 12 h, samples of cecal contents of mice in the control group and the experimental group collected by dissection on the 13th day, stored in disposable samplers, and sent to Chengdu Rhonin Biosciences Co., Ltd. for third-generation sequencing and result analysis.

Diversity analysis of intestinal flora

After obtaining the OTU abundance matrix, alpha and beta diversity analysis were performed to the intestinal flora of the two groups of Kunming mice. Chao1 index was used to measure the species richness, and PD, Shannon-Wiener index and Simpson index were used to measure the species diversity. The analysis was carried out in R language.

Statistical analysis of data

Statistical significance analysis was carried out by SPSS 21.0 software, and the results were expressed by mean ± standard error. Independent sample *T* test was conducted, with $P < 0.05$ as significant and $P < 0.01$ as extremely significant.

Results and Analysis

Effects of compound probiotics on growth performance of Kunming mice

As shown in Table 2, after feeding for 13 d, compared with the blank control group, the weight of Kunming mice in the compound probiotic group increased by 1.93%, and there was no significant difference in weight ($P > 0.05$). However, during the whole experiment, compared with the blank control group, the average daily feed intake (ADFI) and feed-to-weight ratio (F/G) of mice in the compound probiotic group were significantly different ($P < 0.05$), and the ADFI and F/G increased by 13.44% and 7.95%, respectively.

Effects of compound probiotics on immune function of Kunming mice

As shown in Fig. 1, compared with the blank control group, the contents of IgG and IgM in the compound probiotic group increased significantly ($P < 0.05$).

Table 2 Effects of compound probiotics on growth performance of Kunming mice

Variable	Growth performance	
	Control	CB + LR + BS
BW, g		
0 d	11.05 ± 0.05 ^a	11.12 ± 0.81 ^a
5 d	17.50 ± 0.81 ^a	17.78 ± 1.18 ^a
10 d	25.87 ± 1.69 ^a	26.20 ± 2.05 ^a
13 d	29.58 ± 1.61 ^a	30.15 ± 1.53 ^a
0 – 5 d		
ADFI, g/d	3.88 ^a	4.01 ^a
ADG, g/d	1.29 ± 0.15 ^a	1.33 ± 0.15 ^a
F/G	3.01 ^a	3.02 ^a
6 – 10 d		
ADFI, g/d	5.14 ^a	5.56 ^b
ADG, g/d	1.67 ± 0.38 ^a	1.68 ± 0.25 ^a
F/G	3.08 ^a	3.31 ^b
11 – 13 d		
ADFI, g/d	6.37 ^a	8.21 ^b
ADG, g/d	1.24 ± 0.42 ^a	1.32 ± 0.38 ^a
F/G	5.14 ^a	6.22 ^b
0 – 13 d		
ADFI, g/d	4.54 ^a	5.15 ^b
ADG, g/d	1.39 ± 0.12 ^a	1.46 ± 0.11 ^a
F/G	3.27 ^a	3.53 ^b

All the values in the table are mean ± SD ($n=6$). a, b indicates a significant difference between the two groups of data marked with different superscript letters in the same row. CB, *C. butyricum* spores (1×10^{10} cfu/kg); LR, *L. reuteri* (1×10^{11} cfu/kg); BS, *B. subtilis* spores (1×10^{10} cfu/kg); BW, body weight; ADFI, average daily intake; ADG, average daily gain; F/G, feed-to-weight ratio.

Diversity analysis of intestinal flora

As shown in Fig. 2 A – D, Chao1 index and Shannon of the compound probiotics group were significantly lower than those of blank control group ($P < 0.05$). Under the same abundance, a greater Chao1 value indicated more species. The results showed that feeding the compound probiotics diet containing *C. butyricum* spores, *L. reuteri* and *B. subtilis* spores significantly reduced the species diversity of intestinal flora in Kunming mice.

Moreover, the Bray – Curtis distance and Unweighted UniFrac algorithm were used for PCoA analysis and NMDS statistics respectively. As shown in Fig. 2E – F, PCo1 accounted for 33.7% of the total variance, and PCo2 accounted for 13.9% of the total variance. The distance between the compound probiotic group and the blank control group is obviously separated, indicating that the principal components of the flora structures of the two groups were significantly different. In non-metric multi-dimensional scaling (NMDS), Stress = 0.06, less than 0.2, which showed that the grouping and sampling method were reliable.

Composition of intestinal flora and relative abundance analysis of its dominant microorganisms

In order to obtain the abundance information of each taxa at each taxonomic level, based on the OTU abundance table and the annotated taxonomic information table, the data at each taxonomic level, such as Phylum, Genus and Species, were transformed to obtain the relative abundance table of each sample at each taxonomic level. In order to display the average abundance of each group of samples with high abundance (Top20) intuitively, the grouped data were averaged and displayed by histogram (Fig. 3).

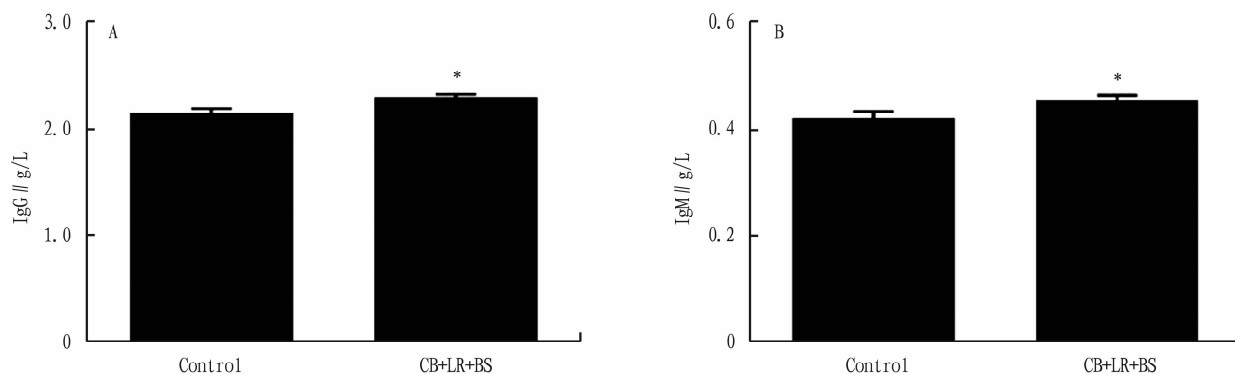


Fig. 1 Effects of compound probiotics on immunoglobulin contents in mice ($\bar{x} \pm s$, $n=6$)

At the phylum level, as shown in Fig. 3A, after 13 d of adding compound probiotics to the basal diet of Kunming mice, compared with the blank control group, the abundance percentages of Firmicutes and Tenericutes increased by 15.04% and 70.47% respectively, while the abundance percentages of Proteobacteria and Bacteroidetes decreased by 22.92% and 27.58% respectively. Therefore, the above results showed that at the phylum level, feeding compound probiotics increased the abundance of Firmicutes and significantly increased the abundance of Tenericutes, while reducing the abundance of Proteobacteria and Bacteroidetes.

At the genus level, as shown in Fig. 3B, compared with the blank control group, the abundance percentage of *Staphylococcus*

in the compound probiotic group increased by 46.82%, and its abundance was relatively high in the cecum of Kunming mice fed with compound probiotics. *Lachnoclostridium*, *Anaerostipes* and *Jeotgalicoccus* were up-regulated by 28.10%, 16.70% and 24.84% respectively. However, *Bacteroides*, *Blautia*, *Acinetobacter*, *Muribaculum* and *Escherichia* were down-regulated by 29.88%, 14.87%, 82.22%, 48.52% and 43.99% respectively. Therefore, at the genus level, feeding compound probiotics (CB + LR + BS) increased the abundance of *Staphylococcus*, *Lachnoclostridium*, *Anaerostipes* and *Jeotgalicoccus*, but decreased the abundance of *Bacteroides*, *Blautia*, *Acinetobacter*, *Muribaculum* and *Escherichia*.

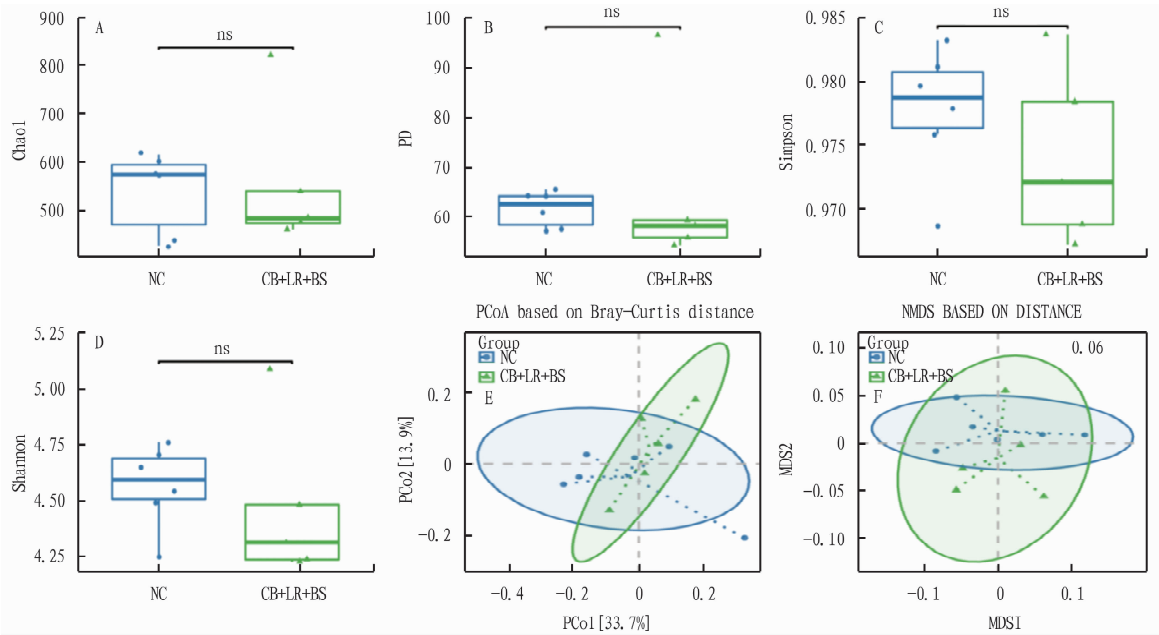


Fig. 2A shows the box plot ($P < 0.05$) of the intergroup difference in Chaol index of microbial communities in the cecal contents of Kunming mice fed in the control group (NC) and compound probiotic experimental group (CB + LR + BS); Fig. 2B shows the box plot of the intergroup difference in Faith's Phylogenetic Diversity (PD) of microbial communities in the cecal contents of Kunming mice fed in the control group and compound probiotic experimental group; Fig. 2C shows the box plot of the intergroup difference in Simpson index of microbial communities in the cecal contents of Kunming mice fed in the control group and compound probiotic experimental group; Fig. 2D shows the box plot of the intergroup difference in Shannon index of microbial communities in the cecal contents of Kunming mice fed in the control group and compound probiotic experimental group; Fig. 2E shows the PCoA plot of microbial communities in the cecal contents of Kunming mice fed in the control group and compound probiotic experimental group; and Fig. 2F shows the NMDS plot of microbial communities in the cecal contents of Kunming mice fed in the control group and compound probiotic experimental group.

Fig. 2 Box plots of intergroup differences of intestinal flora in mice

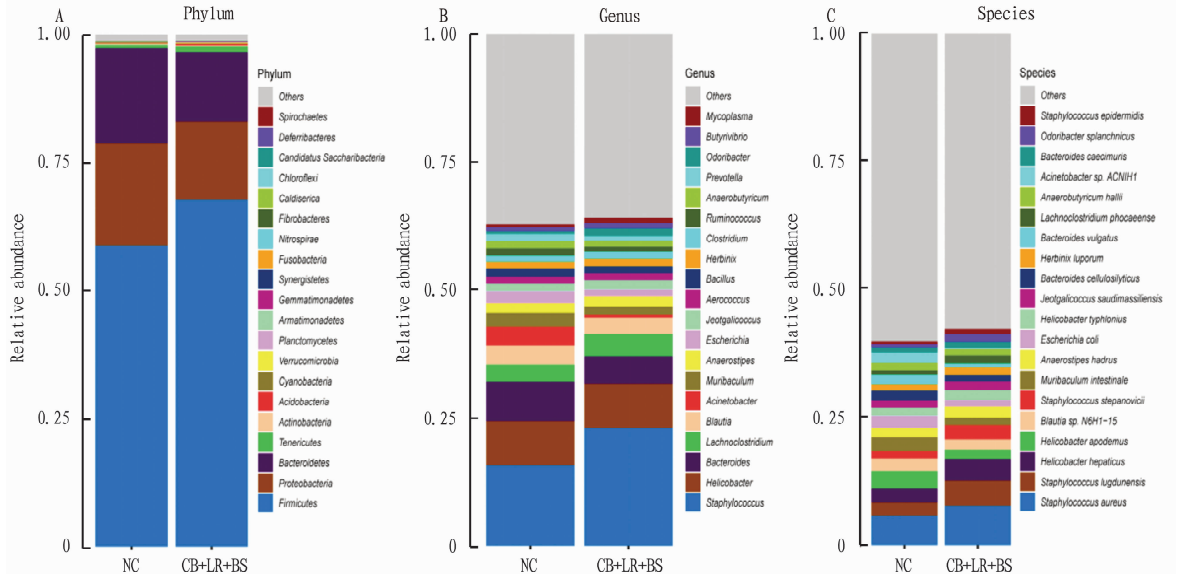


Fig. 3 Histogram of intestinal flora composition in mice

At the species level, as shown in Fig. 3C, compared with the blank control group, the abundance percentages of *Staphylococcus aureus*, *Staphylococcus lugdunensis*, *Helicobacter hepaticus*, *Staphylococcus stepanovicii* and *Anaerostipes hadrus* increased by 34.86%, 92.96%, 53.47%, 95.61%, and 16.70%, respectively. However, the abundance percentages of urease-negative *Helico-*

bacter typhlonius, *Blautia* sp. N6H1-15, *Muribaculum intestinale*, *Escherichia coli* and *Salmonella enterica* decreased by 47.65%, 18.16%, 48.52% and 43.91%, respectively.

In addition, a Sankey diagram (Fig. 4) was drawn. It shows the changing trend of high-abundance species (Top 10) in the compound probiotic group at the level of genus and species respectively.

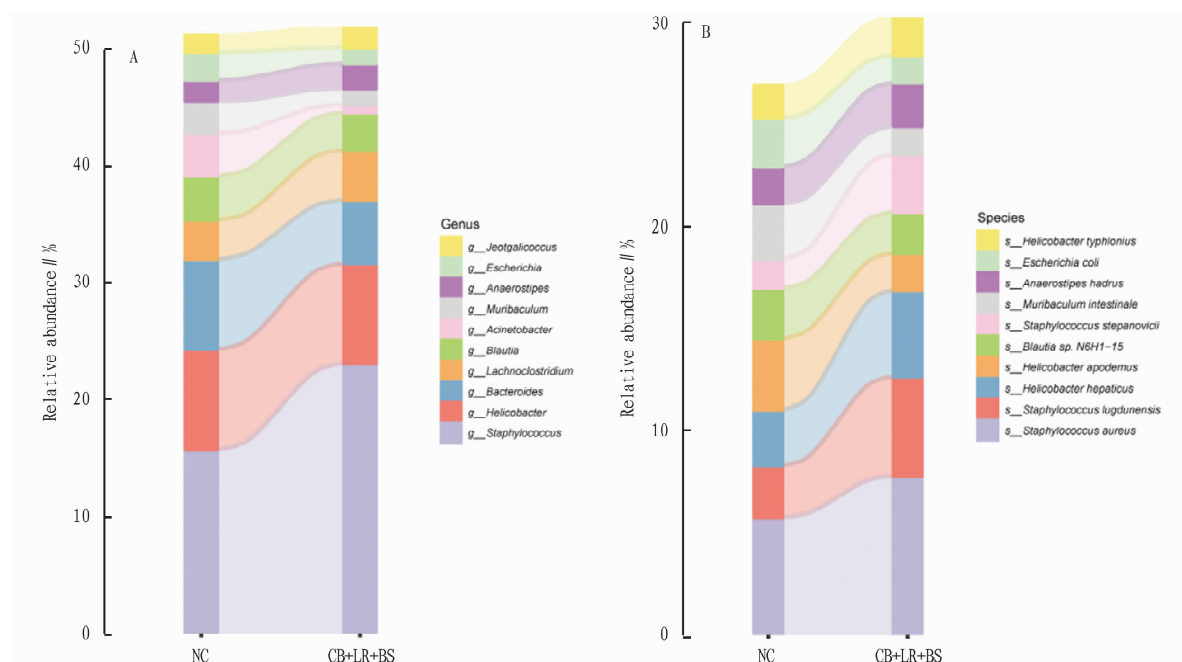


Fig. 4 Sangji diagram of composition of intestinal flora in mice at the genus (A) and species level (B)

Conclusions and Discussion

Effects of compound probiotics on growth performance of Kunming mice

ADFI, ADG and F/G are commonly used indicators for reflecting the growth performance of livestock and poultry. At present, the production performance is improved mainly by improving feed conversion efficiency and utilization rate and reducing feed conversion ratio. Probiotics can maintain the status of probiotics by maintaining the balance of intestinal flora, purifying the intestinal environment and reducing the production of harmful substances, thus improving the growth performance of livestock and poultry. Studies have shown that the compatibility of *B. subtilis* with *C. butyricum* can improve the production performance of laying hens^[4] and the growth performance of broilers^[16].

Wang *et al.*^[17] found that 0.5%, 1.0% and 1.5% compound probiotics (Lactobacillus RSG-1 : *B. subtilis* B-1 : Bacillus licheniformis Y5-39 = 1 : 1 : 4, total viable count $\geq 1.0 \times 10^8$ CFU/g) significantly increased the dry matter intake and average daily weight gain of lambs before and after weaning, thereby improving their growth performance before and after weaning. Zhao *et al.*^[18] found that adding 2.0×10^8 CFU/kg *C. butyricum* and 1.0×10^9 CFU/kg *B. subtilis* to the basal diet significantly increased average daily feed intake and average daily gain of 42-day-old broilers, and reduced feed-to-weight ratio. In this study, *L. reuteri* was added on the basis of *C. butyricum* and *B. subtilis*. After feeding Kunming mice for 13 d, compared with the blank control group, the weight of Kunming mice in the compound probiotic group (CB + LR + BS) increased by 1.93% ($P > 0.05$), the average daily feed intake increased by 13.44% ($P < 0.05$), and the feed-to-weight ratio increased by 7.95% ($P < 0.05$). It showed that the compound probiotics used in the study could improve the growth performance of Kunming mice, but the effect was not significant. In

this study, the growth performance of the compound probiotics group increased slowly, which might be related to feeding time, insufficient management level, animal species and age.

Effects of compound probiotics on immune function of Kunming mice

Immunoglobulin G (IgG) and immunoglobulin M (IgM) are antibody molecules produced by B lymphocytes, which play an important role in the immune response of the body and are important indicators reflecting the immune state of animals. The increase of IgG and IgM in serum indicates the enhancement of immune function.

Zhang *et al.*^[19] found that dietary supplementation with compound probiotics (*B. subtilis* $\geq 4.5 \times 10^9$ CFU/g, *C. butyricum* $\geq 2.0 \times 10^8$ CFU/g) could significantly increase the serum IgM content ($P < 0.05$) and improve the immune function of Lingshan native chickens, and the effect of adding compound probiotics to the diet was slightly better than that of single *C. butyricum*. Li *et al.*^[4] found that adding 1.0×10^{10} CFU/g *B. subtilis* and 1×10^9 CFU/g *C. butyricum* to the diet significantly increased the content of IgG and IgM in laying hens and improved the immune function of laying hens. In this study, *L. reuteri* was added to *C. butyricum* and *B. subtilis*, and it was found that the contents of IgG and IgM in the compound probiotic group increased significantly compared with the blank control group ($P < 0.05$). The results of this study showed that feeding compound probiotics could promote the secretion of immunoglobulin IgG and IgM and improve the immune function of mice.

Effects of compound probiotics on intestinal microflora in Kunming mice

There are a large number of microorganisms in the intestinal tract of livestock and poultry, which can play an important role in the stability of intestinal microecology and the digestion and

absorption of nutrients, thus affecting the growth and development of livestock and poultry. Zhu *et al.* [5] found that adding 3×10^5 CFU/g *C. butyricum* and 5×10^8 CFU/g *B. subtilis* in the diet had a significant effect on the weight, length and microstructure of the intestine in ducks, and it could improve the microstructure of the intestine. Further research by Jie *et al.* [20] found that *C. butyricum* and *B. subtilis* could promote the growth of geese by improving intestinal structure and function, antioxidant capacity and microbial composition. In addition, studies have shown that probiotics can promote the colonization of beneficial bacteria in the intestine, restore destroyed intestinal microflora, and maintain the balance of intestinal microflora, thus alleviating the symptoms of gastrointestinal diseases [21].

In the compound feeding of *C. butyricum*, *L. reuteri* and *B. subtilis*, *B. subtilis* could create an anaerobic environment for the growth of *C. butyricum* [22]. After feeding for 13 d, it was found that compared with the blank control group, in the compound probiotic group, the abundance of harmful bacteria such as *E. coli*, urease-negative *H. typhlonius*, *S. enterica* and *Muribaculum intestinale* decreased significantly, while the abundance of beneficial bacteria such as *A. hadrus* increased significantly, and the abundance of harmful bacteria in *Staphylococcus* such as *S. aureus*, *S. lugdunensis* and *Staphylococcus schleiferi* increased significantly. Therefore, feeding compound probiotic diet can increase the number of beneficial bacteria in the intestines of Kunming mice, reduce the number of harmful bacteria such as *E. coli* and improve the intestinal environment, but the number of harmful bacteria of *Staphylococcus* also increased.

To sum up, feeding the diet supplemented with *C. butyricum*, *L. reuteri* and *B. subtilis* could improve the intestinal microflora of Kunming mice, perfect the intestinal health, significantly reduce the microbial community diversity in the cecal contents of Kunming mice, and improve the immune function of Kunming mice. Feeding compound probiotic diet could effectively reduce the abundance of common harmful bacteria such as *E. coli*, urease-negative *H. typhlonius* and *S. enterica* in the cecal contents of Kunming mice, and increase the abundance of beneficial bacteria such as *A. hadrus*. However, compared with the blank group, the abundance of harmful bacteria in *Staphylococcus* such as *S. aureus*, *S. lugdunensis* and *S. schleiferi* in the compound probiotic group increased significantly.

References

- [1] WANG W. Building a new global competitive advantage of China's probiotic industry with science and technology [N]. China Food News, 2023-06-01(001). DOI:10.28137/n.cnki.ncsp.2023.000883. (in Chinese).
- [2] XIANG XS, YANG RF. Probiotics, prebiotics, metazoans and health [J]. Acta Nutrimenta Sinica, 2023, 45(2): 105–106. (in Chinese).
- [3] GAO KR, MA HR, LI HL, *et al.* The mechanism of probiotics in gastrointestinal: A review [J]. China Food Additives, 2022, 33(5): 194–204. (in Chinese).
- [4] LI AD, XU L, ZHANG MS, *et al.* Effect of *Bacillus subtilis* and *Clostridium butyricum* on production performance, egg quality, serum biochemical and immune indexes of laying hens [J]. Feed Research, 2022, 45(22): 53–56. (in Chinese).
- [5] ZHU CH, LIU HX, SONG WT, *et al.* Effects of dietary *Clostridium butyricum* and *Bacillus subtilis* on intestinal development of Gaoyou ducks [J]. Journal of Anhui Agricultural University, 2023, 50(2): 261–266. (in Chinese).
- [6] YANG TT, LIU MY, ZHANG J, *et al.* The characteristics and biological functions of *Clostridium butyricum* [J]. Feed Industry, 2022, 43(6): 59–64. (in Chinese).
- [7] XU X. Preliminary study on the mechanism of *Clostridium butyricum* improving intestinal mucosal barrier in chickens with necrotic enteritis [D]. Yangling: Northwest A&F University, 2023. (in Chinese).
- [8] HOU CY. Screening and identification of *Clostridium butyricum* in intestine of Jinhua pig based on different body conditions and its effect on improving fat metabolism [D]. Wuhan: Huazhong Agricultural University, 2023. (in Chinese).
- [9] YU XJ, ZHANG MA, HU YJ, *et al.* Isolation, expression and stability analysis of bacteriocins from *Bacillus subtilis* [J]. Acta Veterinaria et Zootechnica Sinica, 2024, 55(1): 323–333. (in Chinese).
- [10] MA ZJ, ZHANG WT, LIAO MD. Effects of *Bacillus subtilis* on growth and development and digestive enzyme activities of *Spodoptera litura* [J]. Southwest China Journal of Agricultural Sciences, 2021, 34(6): 1223–1228. (in Chinese).
- [11] SUI XR. Preparation of antimicrobial substance from *Bacillus subtilis* and its application in aquatic products preservation [D]. Yantai: Yantai University, 2021. (in Chinese).
- [12] ZHENG KT, XU YL, XIONG YF, *et al.* Screening and identification of a cellulase-producing *Bacillus subtilis* from pigs and its effects on growth performance and humoral immunity of piglets [J/OL]. Chinese Journal of Animal Science; 1–12 [2024–02–21]. https://doi.org/10.19556/j.0258-7033.20230417-03. (in Chinese).
- [13] WU LY. Study on functional characteristics of *Lactobacillus reuteri* SH 23 in simulated gastrointestinal fluid culture [D]. Ningbo: Ningbo University, 2022. (in Chinese).
- [14] DUAN YH. Characterization of biological activity of reuterin and construction of its synthetic engineering bacteria [D]. Zhoushan: Zhejiang Ocean University, 2023. (in Chinese).
- [15] The intestinal flora has made contributions to inhibit colorectal cancer through the metabolite reuterin [J]. Practical Oncology Journal, 2022, 36(1): 83. (in Chinese).
- [16] DANG DX, QIANG Q, XU YH, *et al.* Feeding broiler chicks with *Bacillus subtilis*, *Clostridium butyricum*, and *Enterococcus faecalis* mixture improves growth performance and regulates cecal microbiota [J]. Probiotics and antimicrobial proteins, 2022, 16(1): 113–124.
- [17] WANG HY, GUO YX, DUAN CH, *et al.* Effects of compound probiotics on growth performance and weaning stress of lactating lambs [J]. Chinese Journal of Animal Nutrition, 2023, 35(8): 5250–5260. (in Chinese).
- [18] ZHAO X, ZHUANG JR, LI HT, *et al.* Effects of compatibility of *Clostridium butyricum* and *Bacillus subtilis* on growth performance, serum biochemical indices and cecal microflora of broilers [J]. Chinese Journal of Animal Nutrition, 2023, 35(2): 845–854. (in Chinese).
- [19] ZHANG YJ, LI XY, LIAO Y, *et al.* Effect of compound probiotics on growth performance, blood indexes and intestinal morphology of Lingshan chicken [J]. Feed Research, 2023, 46(15): 36–40. (in Chinese).
- [20] JIE Y, BIAO D, MINMENG Z, *et al.* Dietary *Clostridium butyricum* and *Bacillus subtilis* promote goose growth by improving intestinal structure and function, antioxidative capacity and microbial composition [J]. Animals, 2021, 11(11): 3174–3174.
- [21] SONG SC, AN YM, SHIN JH, *et al.* Beneficial effects of a probiotic blend on gastrointestinal side effects induced by leflunomide and amlodipine in a rat model [J]. Beneficial microbes, 2017, 8(5): 801–808.
- [22] ZHANG YR, XIONG HR, GUO XH. Enhanced viability of *Lactobacillus reuteri* for probiotics production in mixed solid-state fermentation in the presence of *Bacillus subtilis* [J]. Folia microbiologica, 2014, 59(1): 31–36.