Supplementation of VLT and marine-derived probiotic BA-9 promotes the growth performance and antioxidant capacity at early life of ruminants

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ABSTRACT

This study aims to investigates the effects of the vine of *Lonicera japonica* Thunb (VLT) and marine-derived Bacillus amyloliquefaciens-9 (BA-9) supplementation on the growth performance, antioxidant capacity, and gut microbiota of goat kids. A total of 32 four-week-old kids were randomly assigned into four groups: a control group (CON), a group supplemented with 0.3% BA-9 (BA-9), a group supplemented with 2% VLT (VLT), and a group supplemented with both 0.3% BA-9 and 2% VLT (MIX). The results indicated that VLT supplementation significantly increased both average daily ($P < 0.001$) and total weight gain ($P < 0.001$), while BA-9 alone had no significant effect ($P > 0.05$) on the average daily and total weight gain. Biomarker analysis of oxidative stress revealed that supplementation of VLT or BA-9 alone enhanced antioxidant capacity. The MIX group showing a higher total antioxidant capacity (T-AOC) compared with the CON, VLT, and BA-9 groups ($P < 0.05$). Plasma albumin levels were significantly increased in the both VLT and BA-9 groups. Microbiota analysis revealed significant differences in α-diversity and β-diversity between the MIX and CON groups, with specific genera such as *Prevotellaceae*_UCG.004 and *Rikenellaceae_RC9*_gut_group negatively correlated with ADG, while *Alistipes* was positively correlated with T-AOC. These findings suggest that the combined supplementation of VLT and BA-9 can significantly enhance growth performance and antioxidant capacity in goat kids by modulating the composition of gut microbiota and reducing oxidative stress.

KEYWORDS: antioxidant capacity; growth performance; goat kids; probiotic;

INTRODUCTION

The young animals are born with immature immune and digestive systems, which makes them highly susceptible to oxidative stress [1]. The incidence of oxidative stress often leads to decreased growth performance, feed efficiency, and survival rates in livestock [2, 3]. A healthy and balanced microbiome is particularly important for the growth, development, and metabolism of ruminants. Specifically, there has been increasing interest in the early development of the gut or rumen microbiome in farm livestock as a means of maintaining health [4, 5]. However, feeding microorganisms alone presents challenges, such as low colonization rates or weak efficiency [6, 7]. Therefore, it is essential to develop feeding additives that can improve the health of young animals, maximizing their overall growth performance.

Marine-derived bioactive compounds and probiotics provide a wide range of health benefits, including antioxidant, immunomodulatory, and anti-inflammatory effects [8–10]. These compounds have significant potential for applications in health supplements. Bacillus amyloliquefaciens-9 (BA-9), which is isolated from the intestinal tract of the white-spotted bamboo shark (*Chiloscyllium plagiosum*), can secrete potential antibacterial materials, such as β-1,3-1,4-glucanase and antimicrobial peptides [11]. Animal feeding experiments have demonstrated that this probiotic reduces the occurrence of diarrhea in goat kids and decreases oxidative stress in the mammary gland by altering the diversity of the intestinal microbial community [12, 13]. Combining probiotics with Chinese herbal polysaccharides has been found to improve the growth performance of lambs and the diversity of rumen bacteria [14]. Recent data combining probiotics with traditional Chinese medicine suggests that this combination has an enhanced effect in inhibiting intestinal inflammatory responses and reducing disease recurrence compared to using probiotics or traditional Chinese

medicine alone [15]. <mark>Combined with plant-derived bioactive compounds provides an ideal strategy to expand the usage of BA-9 in animal feeding.</mark>

Lonicera japonica Thunb (LT) is a widely used traditional Chinese herb with medicinal value attributed to the entire plant. This plant is rich in primary bioactive components including chlorogenic acid and luteolin glucoside. These components have antioxidant and immunomodulatory properties [16–18]. Supplementation of LT extract significantly decreases the respiratory rate in heat-stressed dairy cows and promote their antioxidant and immune functions [19]. However, the use of LT extract is challenged by the high costs of extraction, which can increase production expenses in large-scale applications [20]. Studies suggest that <mark>the vine of *Lonicera japonica* Thunb (VLT)</mark> also exhibits biological activity and can serve as a cost-effective alternative [21].

BA-9 and LT have demonstrated significant antioxidant and health-promoting effects when used individually [22–24]. However, the mechanisms underlying their combined effects remain unclear. BA-9, as a probiotic, may synergize with the bioactive compounds in VLT, such as flavonoids and phenolic acids, to enhance antioxidant capacity, modulate gut microbiota, and reduce oxidative stress more effectively than when used alone [22–24].

This study aims to investigate the dietary addition of VLT and the BA-9 and their interaction on the growth performance and health status of young ruminants using the Nanjiang Yellow <mark>goats</mark> as a model.

2 MATERIALS AND METHODS

2.1 Material Preparation

Bacillus amyloliquefaciens-9 (China General Microbiological Culture Collection

Center number: 13337, accession number: CP021011) was isolated from the intestinal tract of the white spotted bamboo shark (C. *plagiosum*) [25]. The fermentation of Bacillus-9 was prepared following the reported procedure [26]. The Bacillus-9 powder was prepared using a spray dryer (L-217, 1.0 mm nozzle, Lai Heng, Beijing, China). The inlet air temperature, aspirator, liquid flow, and compressed spray airflow were set at 55°C, 2 L/h, and 50 L/h, respectively. Corn starch was used as an adhesion agent at a ratio of 100 g/L. The colony-forming units in the powder were more than 2 $\times 10^9$ /g. The production of BA-9 is gifted from Professor Zhengbing Lv in Zhejiang Sci-tech University.

The dry VLT (produced in Nanjiang County, Sichuan province, China) is ground using a grinder to obtain fine VLT powder. The powder was then sifted through a mesh sieve to remove larger particles, ensuring uniform and fine consistency. The prepared VLT powder was stored in dry, sealed containers.

2.2 Animal Management and Experimental Design

This study was approved by the Experimental Animal Management Committee of Zhejiang University. A total of 32 healthy suckling goat kids (30 $\pm$ 3 days old) with similar body weights (8.68 $\pm$ 0.69 kg) were selected from the Nanjiang Yellow Goat Breeding Farm in Nanjiang County, Sichuan Province, China. Prior to the experiment, the health status of all goat kids was confirmed by veterinary examination. All goat kids were sourced from the same farm to minimize genetic and environmental variation and were uniformly managed during the pre-experimental and experimental periods. Weights were recorded before morning feeding.

During the trial, all goat kids remained in the suckling phase and were not weaned. Natural nursing by their dams was maintained throughout the study, allowing each goat kid to consume milk freely according to its needs without any artificial restrictions. To support growth and gastrointestinal development, a gradually increasing amount of starter feed (pelleted) was introduced. Starter feed was provided twice daily at 8:00 AM and 3:00 PM. The specific feeding regimen was as follows: 100 g/day per kid during weeks 1 to 3, increased to 300 g/day per kid during weeks 4 to 5, and further increased to 500 g/day per kid during weeks 6 to 8. Pellets were evenly distributed at each feeding, and leftover feed was recorded after each meal to monitor feed intake and assess feeding behavior. Efforts were made to ensure adequate feed intake while minimizing wastage.

Using a randomized block design, the goat kids were randomly assigned to one of four groups (n = 8 per group). The groups were as follows: the control group (CON) fed only the basal diet (pellets, provided by Advanced Feed Co., Ltd., Chengdu, China); the BA-9 group, fed the basal diet supplemented with 0.3% BA-9; the VLT group, fed the basal diet supplemented with 2% VLT; and the MIX group, fed the basal diet supplemented with both 0.3% BA-9 and 2% VLT. To ensure consistency in nutrient intake, the feed for each group was thoroughly mixed with the respective supplements and processed into pellets. Water was provided ad libitum, and neck collars were used during feeding to reduce competition for feed.

The nutrient composition of the basal diet is presented in Supplementary Table S1. The experiment consisted of a 1-week adaptation period followed by a 7-week

formal feeding period. Initial body weights were recorded at the start of the trial, and subsequent body weight measurements were conducted every two weeks in the morning before feeding to ensure accuracy and consistency.

Health monitoring was conducted daily throughout the study. Any signs of illness, such as diarrhea, lethargy, or reduced feed intake, were promptly recorded and treated as necessary. No major health issues were observed during the trial. These management practices ensured consistent experimental conditions and reliable outcomes.

2.3 Sample Collection and Analysis

Blood samples were collected from all kids on the last day. Blood was collected from the posterior jugular vein of each kid from 9:00 a.m. to 10:00 a.m. The plasma was separated by centrifugation. The plasma levels of malondialdehyde (MDA), Glutathione peroxidase (GSH-Px), total antioxidant capacity (T-AOC), immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), interleukin-2 (IL-2), interleukin-4 (IL-4) and interleukin-6 (IL-6) were measured using specific commercial kits (Nanjing Jiancheng Biotech, Nanjing, Jiangsu, China) [26]. Fecal samples were collected at last two days at the end of the experiment. The homogenized samples were snap-frozen in liquid nitrogen and stored at −80°C for subsequent DNA analysis.

2.4 16S rRNA Gene Sequencing

Total genomic DNA was extracted from the fecal samples using a commercial kit (Tiangen Biotech, Beijing, China). The V3–V4 regions of the bacterial 16S rRNA

genes were amplified and then paired-end sequenced (2×300 bp) on the Illumina MiSeq platform following standard protocols (Novogene Technology Co., LTD, Tianjing, China).

2.5 Sequencing Data Analysis

Raw reads from different samples were demultiplexed and quality-filtered according to established methods [27]. Bioinformatics analysis was performed using QIIME 2. Shannon and Chao1 indices were calculated to estimate bacterial richness and community diversity [28]. Principal Coordinate Analysis (PCoA) and Linear Discriminant Analysis Effect Size (LEfSe) were conducted using the Novomagic platform (https://magic.novogene.com). Correlation heatmaps were generated using the pheatmap package in R studio. Weighted Gene Co-expression Network Analysis (WGCNA) was performed using the WGCNA package in R studio, and the resulting network was visualized using Cytoscape (Version 3.8.0).

2.6 Statistical Analysis

The data of growth performance was analyzed using a one-way ANOVA with the SPSS software (SPSS v.19, SPSS Inc., Chicago, IL, USA). The statistical analyses of various factors in plasma and microbiol diversity were performed by a one-way ANOVA. Data are presented as means plus SEM. $P < 0.05$ was considered statistically significant. Correlation networks were generated using Spearman's rank correlation coefficients and visualized using the Cytoscape. The significant correlation between bacterial genus and the immune globulins and cytokines was considered when $|R| > 0.2$ and $P < 0.05$).

3 RESULT

3.1 Supplementation of VLT and BA-9 significantly promotes the daily weight gain of goat kids

To investigate the effects of VLT and BA-9 on the growth performance of kids, we first assessed their impact on weight gain. As shown in Table 1, the initial body weight (IBW) of the kids did not differ significantly among the four groups, but there was a trend towards differences in final body weight (FBW) (P = 0.094). Compared with the CON group, feeding goat kids with VLT alone significantly increased their average daily gain (ADG) (P < 0.001) and total weight gain (TWG) (P < 0.001). No significant changes were observed in the BA-9 group. Interestingly, the combined VLT powder and BA-9 (MIX group) outperformed the individual additions (P < 0.001). Compared to the CON group, feeding BA-9 alone did not significantly increase TWG or ADG. However, goat kids in the VLT group showed a significant increase in ADG (P = 0.007), which was further enhanced in the MIX group. Additionally, goat kids in the MIX group had higher average daily weight gain compared to both the CON group (P < 0.001) and BA-9 group (P = 0.014). Furthermore, there was an increasing trend in ADG compared to the VLT group (P = 0.081).

3.2 VLT and BA-9 alter biomarkers in oxidative status and nutrition metabolism

To further investigate the physiological and metabolic mechanisms responsible for the increased daily weight gain in goat kids with the supplementation of VLT, BA-9, or their combination, we examined biomarkers associated with oxidative status and nutrition metabolism. As shown in Table 2, MDA (P = 0.504) and GSH-Px (P = 0.353) activities did not differ significantly among the different treatment groups.

However, the combined supplementation significantly increased the T-AOC of the kids (*P* = 0.039). The single supplementation of VLT (*P* = 0.083) or BA-9 (*P* = 0.084) had minor effects on T-AOC. The MIX group exhibited a significantly higher T-AOC to the CON group (*P* = 0.021), the BA-9 group (*P* = 0.022), and the VLT group (*P* = 0.012). There were no significant changes in triglyceride (*P* = 0.387) and glucose (*P* = 0.661) among the different treatment groups.

3.3 VLT and BA-9 alter biomarkers in immune response

Compared with the CON group, BA-9 (*P* = 0.004) and VLT (*P* = 0.035) significantly increased the plasma albumin (ALB) level. There was an observed increasing trend in the MIX group compared with the CON group (*P* = 0.081). There were no significant changes in IgA (*P* = 0.115), IgG (*P* = 0.387), IgM (*P* = 0.257), IL2 (*P* = 0.950), IL4 (*P* = 0.446), and IL6 (*P* = 0.880).

3.4 Supplementation of VLT and BA-9 decreases the diversity indices of fecal microbiota

To investigate the combined effects of VLT powder and BA-9 in goat kids, the fecal microbiota was assessed using 16S rRNA sequencing. The amplicon sequencing data were assessed using rarefaction curves. These curves reached a plateau as the sample size increased, indicating that the species distribution within the samples was even and that we had sufficient sequencing depth to cover the major species (Figure S1). This outcome provides a reliable basis for our data and ensures the accuracy of subsequent analyses. We classified all fecal microbiota into 6214 ASVs through amplicon sequencing of the 16S rRNA gene. The Venn diagram illustrates that there were 552 unique ASVs in the CON group, 384 in the BA-9 group, 1072 in the VLT

group, and 1071 in the MIX group, with 1173 ASVs shared among all four groups (Figure 1A). We used the Chao1, Simpson, and Shannon indices to examine the α-diversity of the fecal microbiota (Figure 1B-D). Compared with the CON group, the MIX group showed significantly decreased Chao1 ($P < 0.001$), Simpson ($P < 0.001$), and Shannon ($P < 0.001$) indices, indicating a lower diversity of microbial species (Figure 1B-D). We also found significant differences in α-diversity indices between the MIX group and the BA-9 and VLT groups for these indices ($P < 0.001$). There were no significant differences in Chao1, Simpson and Shannon indices when the diet was supplemented with BA-9 or VLT alone compared to the CON group. PCoA based on unweighted UniFrac distances showed distinct clustering of samples based on the different treatments (PCoA1 = 28.92%, PCoA2 = 9.93%) (Figure 1E).

3.5 Comparison of fecal microbial profiling between CON and MIX groups

To further explore the combined effects of combination VLT and BA-9 on kids, the microbiota profiling between the MIX and CON groups was compared. At the phylum level, the MIX group showed higher abundances of *Verrucomicrobiota* ($P = 0.007$) and *Patescibacteria* ($P = 0.037$) compared to the CON group (Figure 2A). Additionally, the MIX group had lower abundances of *Campylobacterota* ($P = 0.029$) and *Desulfobacterota* compared to the CON group ($P = 0.008$). At the genus level, the abundances of *Akkermansia* ($P = 0.006$), *Candidatus_Stoquefichus* ($P = 0.008$), *Faecalibacterium* ($P = 0.048$), *Candidatus_Saccharimonas* ($P = 0.038$) and *UBA1819* ($P = 0.004$) were significantly increased in the MIX group. Conversely, the abundances of *Rikenellaceae_RC9_gut_group* ($P = 0.001$), *Campylobacter* ($P = 0.035$), *Turicibacter* ($P = 0.009$), *Desulfovibrio* ($P = 0.005$), *NK4A214 group* ($P = 0.019$), *Clostridium_sensu_stricto_1* ($P = 0.008$), *Ruminococcus* ($P = 0.009$),

Romboutsia (*P* = 0.011), and *Defluviitaleaceae_UCG_011* (*P* = 0.030) were significantly decreased in MIX group compared to the CON group (Figure 2B). At the species level, the MIX group had higher abundances in *Bacteroides_massiliensis* (*P* = 0.007), *Akkermansia_muciniphila* (*P* = 0.022)*, Alistipes_finegoldii* (*P* = 0.011), *Parabacteroides_merdae* (*P* = 0.029) and *Bacteroides_caecigallinarum* (*P* = 0.010) but a lower level of *Romboutsia_sp_DR1* (*P* = 0.011) compared to the CON group (Figure 2C). Furthermore, the <mark>LEfSe</mark> further identified differential abundances in bacterial taxa between the MIX and CON groups. The MIX group was enriched with o_*Pseudomonadales*, f_*Moraxellaceae*, g_*Acinetobacter*, g_*Bacteroides*, f_*Bacteroidaceae*, and f_*Muribaculaceae*, while the CON group was enriched with f_*Barnesiellaceae*, g_*Prevotellaceae_UCG_004*, g_*Rikenellaceae_RC9_gut_group*, and f_*Rikenellaceae* in When LDA was greater than 4. (Figure 2D-E).

3.6 Hub microbiota correlated with growth performance and antioxidant capacity

Spearman correlation analysis was conducted to determine the correlation between fecal microbiota and ADG, T-AOC and ALB at the genus level (Figure 3A). The results showed that three genera in feces including *Prevotellaceae_UCG_004* (*P* < 0.01, R = -0.77), *UCG_005* (*P* < 0.01, R = -0.53) and *Rikenellaceae_RC9_gut_group* (*P* < 0.01, R = -0.58) were negatively correlated with ADG. *Alistipes* was positively correlated with T-AOC (*P* < 0.01, R = 0.76), while *Blautia* was negatively correlated with T-AOC (*P* = 0.03, R = -0.44).

The correlation between ALB, T-AOC or ADG and the microbial abundance at the genus level was further investigated using WGCNA. A total of six microbiota modules were identified (Figure 3B). The MEblue was significantly associated with

ADG (P = 0.035, R = -0.399), the MEyellow was significantly associated with ADG (P = 0.02, R = -0.437) and MEyellow was significantly associated with T-AOC (P = 0.043, R = -0.385). The network derived from the MEblue module indicated that o_*Bacteroidales_RF16_group*, g_*Clostridium_sensu_stricto_1*, f_*Peptostreptococcaceae* and g_*Romboutsia* were the hub microbiota within the MEblue module (Figure 3C). The network derived from the MEyellow module indicated that g_*Desulfovibrio*, *Spirochaetes_bacterium_GWE2_31_10*, and *Lachnospiraceae_NK4B4_group* were the hub microbiota within the MEyellow module (Figure 3C).

4 DISUSSION

Young livestock are highly susceptible to various diseases because of their immature antioxidant and immune systems, which leads to a low rate of growth performance [29–31]. This study aims to optimize rearing strategies for goat kids by promoting the status of their antioxidant and immune systems through the addition of BA-9 and VLT. Our data found that VLT and BA-9 exhibited significant synergistic effects in promoting the growth performance of goat kids by enhancing the total antioxidant capacity. Furthermore, the association analysis demonstrated that combined administration of VLT and BA-9 modulates the fecal microbial community of kids, thereby optimizing their physiological functions and health status.

Young kids, due to their immature digestive and immune systems, are susceptible to environmental changes, often resulting in a low average daily weight gain [12]. The increase in average daily weight gain in the VLT group is consistent with previous data in Saanen Kids [31]. It is worth noting that when both VLT and BA-9 were added to the kids' feed, the weight gain effect was superior to that of adding either alone.

This indicates a synergistic effect in promoting the growth of kids. One possible reason for this is that chlorogenic acid in *Lonicera japonica* Thunb increases transepithelial electrical resistance and reducing horseradish peroxidase flux, which shows potential in repairing the intestinal barrier and maintaining intestinal health [32]. Moreover, BA-9 derived from marine organisms contains various antimicrobial peptides [13]. These peptides can enhance the bioavailability of *Lonicera japonica* Thunb by altering the metabolic functions of the gut microbiota or directly improving the overall function of the intestine in young goats. This idea of a synergistic interaction between VLT and BA-9 is further supported by the effects of VLT in promoting the growth of kids. Our data indicate that adding both VLT and BA-9 provides an ideal strategy for promoting the health of young goats.

Our study found that adding VLT and BA-9 to the diet did not impact total protein, triglycerides or blood glucose levels, indicating that these additives do not influence kids' nutritional metabolism. However, when VLT and BA-9 were added simultaneously, there was a significant increase in plasma ALB levels in goat kids. This suggests that there is a synergistic effect between these two additives, which may involve complex interactions between intestinal immune regulatory cells and immune signaling molecules. This provides new insights into the regulation of immune function in goat kids [32].

During nutritional metabolism, an amount of reactive oxygen species (ROS) are produced, which can damage cells and tissues and cause oxidative status [34]. T-AOC (total antioxidant capacity) and GSH-Px activity are key markers used to assess the antioxidant capacity [34]. The current study showed that adding VLT or BA-9 alone did not affect the antioxidant capacity of goat kids. However, when VLT and BA-9 were fed together, there was a significant increase in total antioxidant capacity of kids.

This suggests that this combination has an antioxidant activity. VLT and BA-9 may interact to enhance the absorption and utilization of antioxidant components in *Lonicera japonica* Thunb, thereby further enhancing its antioxidant effects.

The observation of a positive correlation between *Alistipes* and T-AOC agrees with the fact that *Alistipes* improves gut health and enhances the host's antioxidant capacity through its metabolic products, such as short-chain fatty acids [35]. WGCNA revealed microbiome modules significantly associated with ADW and T-AOC, particularly the MEblue and MEyellow modules. The central species within these modules, such as *Clostridium_sensu_stricto_1* and *Desulfovibrio*, may play crucial roles in influencing the growth performance and antioxidant status of kids by modulating the gut microenvironment and affecting host nutrient absorption and energy metabolism. The activity of *Desulfovibrio*, related to sulfate reduction, impacts the redox state of the gut, thereby influencing the host's antioxidant capacity [36]. The correlation between the microbiota and antioxidants provides an insight into the interactions between microbiota and growth performance as well as antioxidant status in kids. Future research should further explore the specific mechanisms of action of VLT and BA-9, particularly how they modulate the composition and metabolic activities of the gut microbiota. These data may provide evidence for microbiome-based interventions to optimize livestock growth performance during the early stage of life.

5 CONCLUSION

Developing additives that improve the health of young animals can maximize their overall growth performance and productivity throughout their lives. In the current study, our data firstly underscore the significant improvement in growth

performance and antioxidant capacity of goat kids through the combined supplementation of VLT and marine-derived BA-9. This synergistic effect is likely attributed to the modulation of the gut microbiota, as evidenced by significant differences in microbial diversity and the presence of specific bacterial genera that are correlated with growth and antioxidant indices. These findings highlight the potential of using these novel feed additives to enhance the health and productivity of young ruminants, providing a promising strategy for improving livestock rearing practices. Further research should explore the underlying mechanisms of this synergistic interaction to optimize its application in animal husbandry.

Data availability statement

The datasets presented in this study were deposited in the NCBI Sequence Read Archive (SRA) under the accession PRJNA1143899.

Author contributions

JK: data curation, and writing—original draft. JZ, KL, JW, TL, YC, TL and BM: data curation. HS, JL and JZ: funding acquisition and supervision, and editing draft. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare there are no conflicts of interest.

REFERENCES

1. Shah AM, Cai Y, Zou H, et al (2019) Effects of supplementation of branches and leaves trimmed from tea plant

on growth performance, rumen fermentation and meat composition of Nanjiang Yellow Goats. ANIMALS, 9(9), 590.

2. Du W, Wang X, Hu M, et al (2023) Modulating gastrointestinal microbiota to alleviate diarrhea in calves. FRONTIERS IN MICROBIOLOGY, 14, 1181545.

3. Du W, Wang X, Xu L, et al (2023) Editorial: research advances in intestinal diseases and related diarrhea in animal production. FRONTIERS IN VETERINARY SCIENCE, 10, 1201231.

4. Balasubramanian B, Liu W-C (2024) Editorial: gut microbiota: allied with livestock nutrition, health, and welfare. FRONTIERS IN VETERINARY SCIENCE, 11, 1413671.

5. Wang Y, Guan LL (2022) Translational multi-omics microbiome research for strategies to improve cattle production and health. EMERGING TOPICS IN LIFE SCIENCES, 6(2), 201–213.

6. Yadav M, Kumar S, Parsana Y, et al (2024) Non-encapsulated, encapsulated, and lyophilized probiotic limosilactobacillus reuteri SW23 influenced the growth and gut health in calves. SCIENTIFIC REPORTS, 14, 7657.

7. Ban Y, Guan LL (2021) Implication and challenges of direct-fed microbial supplementation to improve ruminant production and health. JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY, 12, 109.

8. Šimat V, Elabed N, Kulawik P, et al (2020) Recent advances in marine-based nutraceuticals and their health benefits. MARINE DRUGS, 18(12), 627.

9. Babbar R, Kaur A, Vanya null, et al (2024) Impact of bioactive compounds in the management of various inflammatory diseases. CURRENT PHARMACEUTICAL DESIGN, 30(24), 1880-1893.

10. Shahidi F, Ambigaipalan P. (2015) Novel functional food ingredients from marine sources. CURRENT OPINION IN FOOD SCIENCE, 2, 123–129.

11. Zhang W, Wei L, Xu R, et al (2020) Evaluation of the antibacterial material production in the fermentation of Bacillus amyloliquefaciens-9 from whitespotted Bamboo Shark (Chiloscyllium plagiosum). MARINE DRUGS, 18(2), 119

12. Zhang W, Xin H, Jiang N, et al (2021) Bacillus amyloliquefaciens-9 as an alternative Approach to Cure Diarrhea in Saanen Kids. ANIMALS, 11(3), 592.

13. Li Y, Jiang N, Zhang W, et al (2021) Bacillus amyloliquefaciens-9 reduces somatic cell count and modifies fecal microbiota in lactating goats. MARINE DRUGS, 19(8), 404.

14. Chen H, Guo B, Yang M, et al (2021) Response of growth performance, blood biochemistry indices, and rumen bacterial diversity in lambs to diets containing supplemental probiotics and Chinese medicine polysaccharides. FRONTIERS IN VETERINARY SCIENCE, 8, 681389.

15. Hu Y, Ye Z, She Y, et al (2022) Efficacy and safety of probiotics combined with traditional Chinese medicine for ulcerative colitis: a systematic review and meta-analysis. FRONTIERS IN PHARMACOLOGY, 13, 844961.

16. Shang X, Pan H, Li M, et al (2011) *Lonicera japonica* Thunb.: ethnopharmacology, phytochemistry and pharmacology of an important traditional Chinese medicine. JOURNAL OF ETHNOPHARMACOLOGY, 138(1), 1–21.

17. Zheng S, Liu S, Hou A, et al (2022) Systematic review of Lonicerae Japonicae Flos: a significant food and traditional Chinese medicine. FRONTIERS IN PHARMACOLOGY, 13, 1013992.

18. Guo Y-P, Lin L-G, Wang Y-T (2015) Chemistry and pharmacology of the herb pair Flos Lonicerae japonicae-Forsythiae fructus. CHINESE MEDICINE, 10, 16.

19. Ma FT, Shan Q, Jin YH, et al (2020) Effect of *Lonicera japonica* extract on lactation performance, antioxidant status, and endocrine and immune function in heat-stressed mid-lactation dairy cows. JOURNAL OF DAIRY SCIENCE, 103(11), 10074-10082.

20. Cannavacciuolo C, Pagliari S, Celano R, et al (2024) Critical analysis of green extraction techniques used for botanicals: Trends, priorities, and optimization strategies-a review. TRAC TRENDS IN ANALYTICAL CHEMISTRY, 173, 117627.

21. Cai Z, Wang C, Zou L, et al (2019) Comparison of multiple bioactive constituents in the flower and the caulis of lonicera japonica based on UFLC-QTRAP-MS/MS combined with multivariate statistical analysis. MOLECULES, 24(10), 1936.

22. Liu T, Li F, Xu J, et al (2022) Metabolome analysis reveals potential mechanisms of mannan oligosaccharides to improve health, growth performance, and fatty acid deposition in hu lambs. AGRICULTURE, 12(9), 1327.

23. Xiong Y, Guo C, Wang L, et al (2021) Effects of paper mulberry silage on the growth performance, rumen microbiota and muscle fatty acid composition in hu lambs. FERMENTATION, 7(4), 286.

24. Liu T, Bai Y, Wang C, et al (2023) Effects of probiotics supplementation on the intestinal metabolites, muscle fiber properties, and meat quality of sunit lamb. ANIMALS, 13(4), 762.

25. Wu J, Xu G, Jin Y, et al (2018) Isolation and characterization of Bacillus sp. GFP-2, a novel Bacillus strain with antimicrobial activities, from Whitespotted bamboo shark intestine. AMB EXPRESS, 8, 84.

26. Kritas SK, Govaris A, Christodoulopoulos G, Burriel AR (2006) Effect of Bacillus licheniformis and Bacillus subtilis supplementation of ewe's feed on sheep milk production and young lamb mortality. JOURNAL OF VETERINARY MEDICINE SERIES A, 53(4), 170–173.

27. Zhong Y, Xue M-Y, Sun H-Z, et al (2020) Rumen and hindgut bacteria are potential indicators for mastitis of mid-lactating holstein dairy cows. MICROORGANISMS, 8(12), 2042.

28. Bolyen E, Rideout JR, Dillon MR, et al (2019) Author correction: reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. NATURE BIOTECHNOLOGY, 37(9), 1091.

29. Ma J, Wang C, Wang Z, et al (2021) Active dry yeast supplementation improves the growth performance, rumen fermentation, and immune response of weaned beef calves. ANIMAL NUTRITION, 7(4), 1352-1359.

30. Colditz IG, Watson DL, Gray GD, et al (1996) Some relationships between age, immune responsiveness and

resistance to parasites in ruminants. INTERNATIONAL JOURNAL FOR PARASITOLOGY, 26(8), 869–877.

31. Long Y, Xiao W, Zhao Y, et al (2024) Effects of flammulina velutipes mushroom residues on growth performance, apparent digestibility, serum biochemical indicators, rumen fermentation and microbial of Guizhou black goat. FRONTIERS IN MICROBIOLOGY, 15, 1347853.

32. Wang J-H, Bose S, Kim G-C, et al (2014) Flos Lonicera ameliorates obesity and associated endotoxemia in rats through modulation of gut permeability and intestinal microbiota. PLOS ONE, 9(1), e86117.

33. Rahmani AH, Almatroudi A, Allemailem KS, et al (2024) oleuropein, a phenolic component of olea europaea l. ameliorates ccl4-induced liver injury in rats through the regulation of oxidative stress and inflammation. EUROPEAN REVIEW FOR MEDICAL AND PHARMACOLOGICAL SCIENCES, 28(4), 1259–1271.

34. Wang Y, Liu J, Xiao H, et al (2024) Dietary intakes of vitamin D promote growth performance and disease resistance in juvenile grass carp (Ctenopharyngodon idella). FISH PHYSIOLOGY AND BIOCHEMISTRY, 50(3), 1189–1203.

35. Aljumaah MR, Bhatia U, Roach J, et al (2022) The gut microbiome, mild cognitive impairment, and probiotics: A randomized clinical trial in middle-aged and older adults. CLINICAL NUTRITION, 41(11), 2565–2576.

36. Chen Y, Lu J, Zhu S-M, et al (2020) Effect of gegen qinlian decoction and it's different compatibility groups on gut microbiota in rats with acute enteritis based on high-throughput sequencing. ZHONGGUO ZHONG YAO ZA ZHI, 45(6), 1406–1417.

Table 1. Effects of BA-9 and VLT on the growth performance of Nanjiang Yellow Goat kids

Items[2]	Groups				SEM	*p* Value
	CON[3]	BA-9[3]	VLT[3]	MIX[3]		
IBW, kg	8.26±1.46	8.80±1.94	7.85±5.37	9.79±5.67	0.005	0.311
FBW, kg	10.29±2.19	11.34±4.16	10.9±5.12	13.28±1.94	0.009	0.094
TWG, kg/hd	2.03±4.68[c]	2.54±5.02[bc]	3.05±3.89[ab]	3.49±5.93[a]	0.007	<0.001
ADG, g	65.32±2.64[c]	81.86±2.90[bc]	98.39±4.84[ab]	112.50±2.42[a]	0.004	<0.001

Notes：

[1] The data are expressed as the mean±SEM, *n* = 8 per group .

[a,b,c] Means without a common superscript differ significantly between the two groups at the same time point ($P < 0.05$).

[2] IBW, initial body weight (weight of a seven-day-old lamb); FBW, final body weight (weight of a fifty-day-old lamb); TWG, total weight gain (the difference between FBW and IBW); ADG, average daily gain.

[3] CON, control group; BA-9, BA-9 probiotic supplementation group; VLT, VLT powder supplementation group; MIX, combined supplementation group of both VLT powder and BA-9 probiotic.

Table 2. Effects of BA-9 and VLT on indicators of oxidative status and biochemical indices in Nanjiang Yellow Goat kids

Items[2]	Groups				SEM	p Value
	CON[4]	BA-9[4]	VLT[4]	MIX[4]		
Oxidative Stress[2]						
GSH-Px, U/ml	384.49±3.82	420.61±5.42	363.06±1.95	382.65±2.36	11.99	0.353
MDA, nmol/ml	2.99±2.71	3.20±1.83	3.48±5.02	3.12±6.84	0.21	0.504
T-AOC, mmol/L	2.20±5.75[b]	2.20±2.76[b]	2.18±1.74[b]	2.40±3.35[a]	0.05	0.039
Nutrition Metabolism[3]						
TG, mmol/L	0.93±2.88	0.77±0.73	0.86±1.36	0.75±0.32	0.04	0.387
GLU, mmol/L	5.02±1.41	5.21±1.93	4.93±2.21	5.09±2.52	0.06	0.661
TP, g/L	69.70±2.05	71.46±1.11	67.77±2.60	76.51±5.81	1.87	0.084

Notes:

[1] The data are expressed as the mean±SEM, n = 8 per group.

[a,b,c] Means without a common superscript differ significantly between the two groups at the same time point ($P < 0.05$).

[2] GSH-Px, glutathione peroxidase; MDA, malondialdehyde; T-AOC, total antioxidant capacity.

[3] TG, triglyceride; GLU, glucose; TP, totol protein.

[4] CON, control group; BA-9, BA-9 probiotic supplementation group; VLT, VLT powder supplementation group; MIX, combined supplementation group of both VLT powder and BA-9 probiotic.

Table 3. Effects of BA-9 and VLT on immune indices of Nanjiang Yellow Goat kids

Items[2]	Groups				SEM	*p* Value
	CON[3]	BA-9[3]	VLT[3]	MIX[3]		
IgA, mg/mL	2.35±0.19	2.38±5.33	2.25±0.45	1.91±2.66	0.11	0.115
IgG, mg/mL	10.89±7.50	10.55±4.41	9.49±1.53	9.90±3.30	0.32	0.387
IgM, mg/mL	3.00±1.14	2.82±1.08	2.69±4.93	2.45±2.31	0.12	0.257
IL2, pg/mL	47.07±3.27	48.30±0.05	49.87±3.52	49.67±3.26	0.67	0.950
IL4, pg/mL	79.02±1.88	68.81±0.52	75.35±3.23	71.96±0.89	2.20	0.446
IL6, pg/mL	104.21±6.92	102.59±4.91	98.67±3.48	98.85±3.76	1.38	0.880
ALB, g/L	25.34±4.57[c]	28.56±5.78[a]	27.63±5.50[ab]	26.14±4.23[b]	0.72	0.019

Notes:

[1] The data are expressed as the mean±SEM, *n* = 8 per group.

[a,b,c] Means without a common superscript differ significantly between the two groups at the same time point ($P < 0.05$).

[2] ALB, Albumin.

[3] CON, control group; BA-9, BA-9 probiotic supplementation group; VLT, VLT powder supplementation group; MIX, combined supplementation group of both VLT powder and BA-9 probiotic.
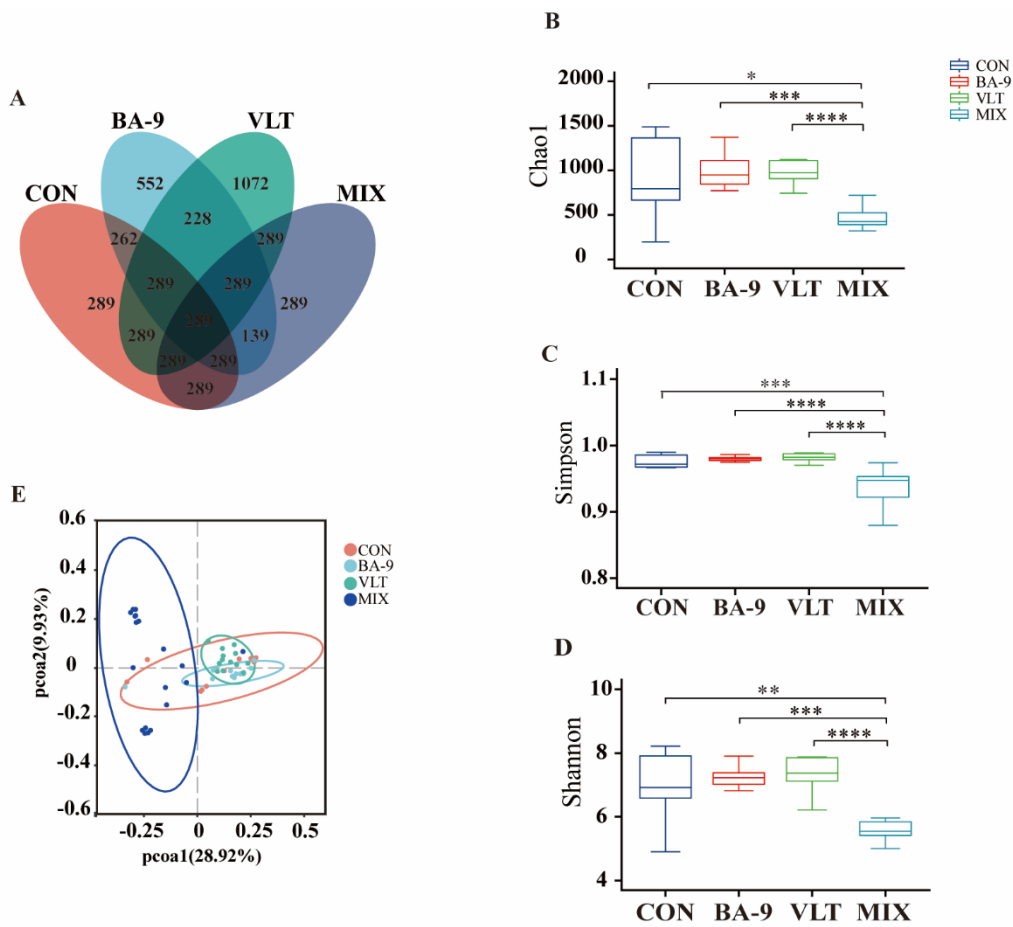
Figure 1

Figure 1. Diversity analysis of fecal microbiota in Nanjiang Yellow Goat kids. (A) Venn diagram of OTUs. (B) Chao 1 index comparing α-diversity among CON, BA-9, VLT and MIX treatment groups. (C-D) Simpson index and Shannon index further confirm significant differences in α-diversity among the groups. (E) PCoA analysis demonstrating intergroup differences in β-diversity. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.
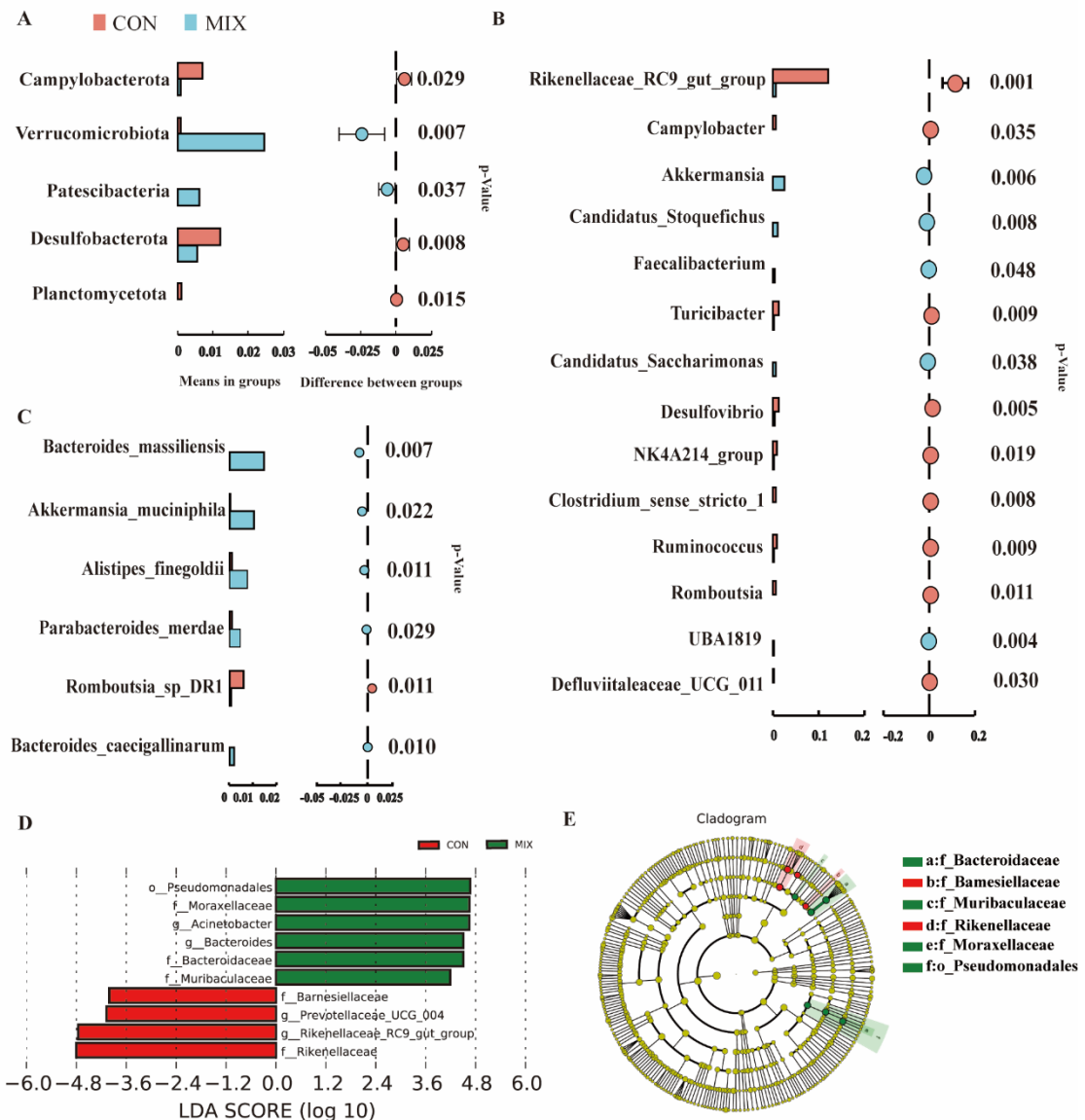
Figure 2

Figure 2 Analysis of the microbial community structure in the CON and MIX groups. (A) Displays the differential bacteria at the phylum level. (B) Displays the differential bacteria at the genus level. (C) Displays the differential bacteria at the species level. (D) Shows the key differential bacteria through Linear Discriminant Analysis (LDA) score plot. (E) The partial phylogenetic tree (Cladogram) reveals the phylogenetic relationships of various microbial categories and their changes in different treatment groups.
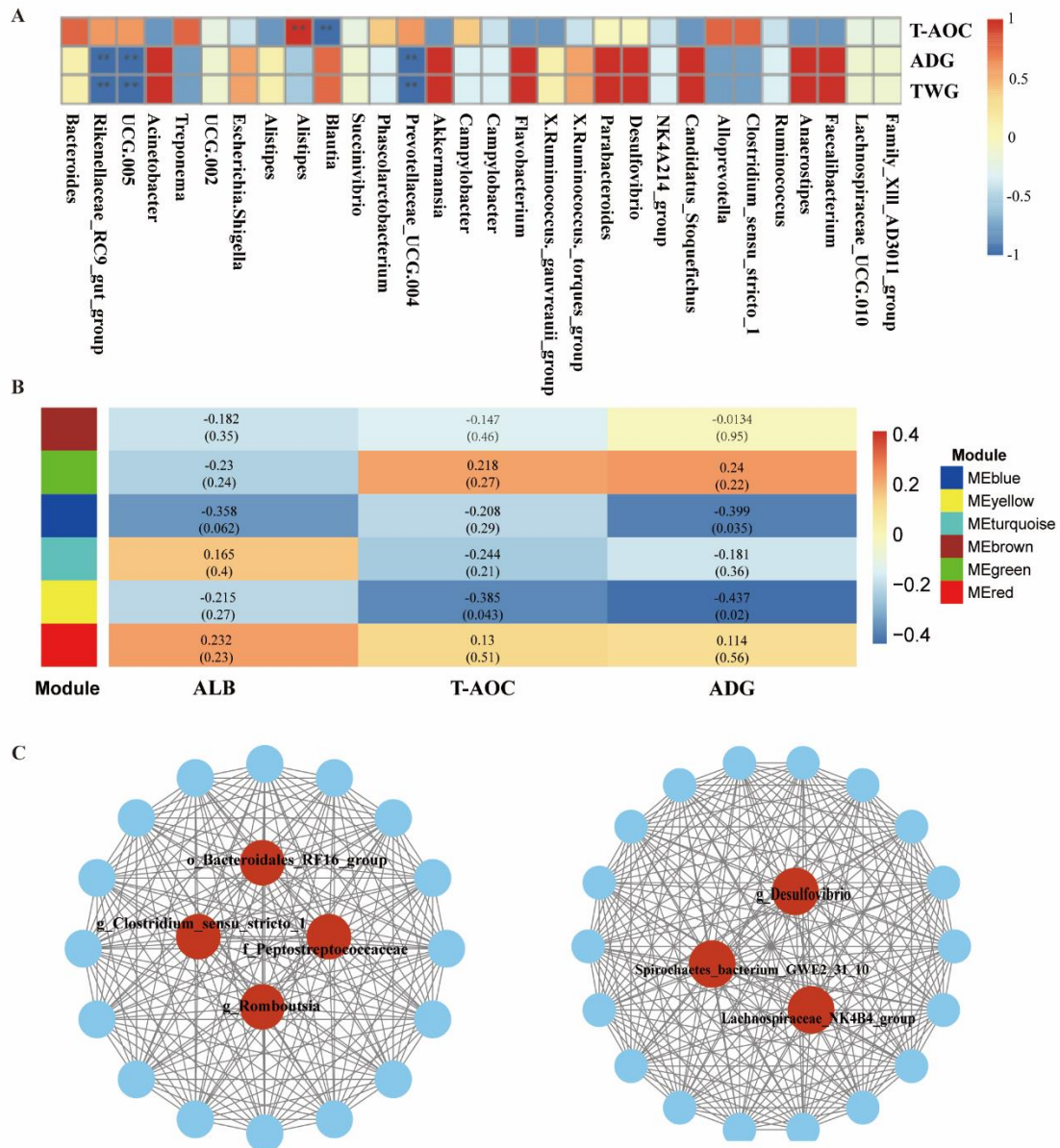
Figure 3

Figure 3 (A) Correlation between environmental factors and fecal bacteria at the genus level, with * *P* < 0.05 and ** *P* < 0.01. Weighted Gene Co-Expression Network Analysis (WGCNA) was used to analyze the related modules. Correlation networks were generated using Spearman's rank correlation coefficient. (B) Heatmap of the WGCNA modules. (C) Interaction network and hub microbes in the MEblue and MEyellow modules.