



Article

Dietary Energy Levels Impact on Skin Microbiota and Metabolites of Yaks

Pengcheng Zhao , Bingang Shi, Xuelan Zhou, Zhidong Zhao , Jiang Hu * and Xiaolan Zhang *

Gansu Key Laboratory of Herbivorous Animal Biotechnology, College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China; zpc15390649768@163.com (P.Z.); shibg@gsau.edu.cn (B.S.); 14201@gsau.edu.cn (X.Z.); zhaozd@gsau.edu.cn (Z.Z.)

* Correspondence: huj@gsau.edu.cn (J.H.); zhangxiaolan@gsau.edu.cn (X.Z.)

Abstract

The study aims to investigate the skin microbiome composition of Yaks and the effects of different dietary nutrient levels on the skin microbiome diversity and metabolites. A total of 19 healthy Tianzhu White Yaks at two age stages (2.5 and 4.5 years old) were selected and fed either a high-energy diet ($n = 9$) or a low-energy diet ($n = 10$). After 90 days of feeding, skin microbiota and skin tissue metabolites were detected using 16S rRNA sequencing and LC-MS/MS untargeted metabolomics, respectively. The results showed: (1) the phyla Firmicutes, Actinobacteriota, Proteobacteria, and Bacteroidetes exhibited relatively high abundances in the skin of yaks, and the total abundance of these four phyla reached as high as 99.3%. Alpha diversity analysis indicated that the alpha diversity index of yak skin microbiota was significantly higher ($p < 0.05$) in the low-energy nutritional level group than in the high-energy nutritional level group in yaks of both 2.5 and 4.5 years of age. Principal coordinate analysis (PCoA) revealed a distinct separation of all skin microbiota samples into two clusters: the high-energy (H) and low-energy (L) groups. (2) A total of 114 differentially expressed metabolites were screened across both groups, significantly enriched ($p < 0.05$) in pathways including synaptic vesicle trafficking and glycerophospholipid metabolism; (3) Correlation analysis between microbiota and metabolites revealed significant positive correlations ($p < 0.01$) between *Psychrobacter* and choline, and between *Corynebacterium* and palmitic acid. In conclusion, A low-energy diet increases skin microbial diversity, which is beneficial for maintaining community stability; In contrast, a high-energy diet enriches bacterial genera such as *Corynebacterium* and *Psychrobacter*, enhancing functions related to antibacterial activity and barrier protection.

Keywords: skin of yaks; diets and nutrition; microbial diversity; differential metabolites



Academic Editor: Todd Riley Callaway

Received: 5 January 2026

Revised: 9 February 2026

Accepted: 10 February 2026

Published: 13 February 2026

Copyright: © 2026 by the authors.

Licensee MDPI, Basel, Switzerland.

This article is an open access article distributed under the terms and conditions of the [Creative Commons Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

Yak has evolved remarkable adaptability to extremely high-altitude environments characterized by severe cold, hypoxia, and intense ultraviolet radiation, as evidenced by long-term habitation [1]. As the largest organ in mammals, the skin comprises the epidermis, dermis, and subcutaneous tissue, along with appendages such as hair follicles, sweat glands, and sebaceous glands that perform diverse physiological functions [2]. These multi-layered structures and appendages enable the skin to regulate body temperature, resist external stresses, counter microbial invasion, maintain water and electrolyte balance, and facilitate tactile perception [3]. The biological functions of skin tissue are closely intertwined with the complex microbial community that colonizes its surface [4]. A healthy skin

microbiome helps maintain ecological balance and defends against pathogenic invasion [5]. Under long-term stress in cold and high-altitude environments, the yak skin microbiota may play a more significant role in adapting to extreme conditions by regulating host immune and metabolic processes, correlating with health status and stress resistance [6]. extremophiles, with their diversity and abundance. At present, due to the complexity of the skin microbiome and limitations of analytical techniques, research on the skin microbiome still faces significant challenges. McBride M. E. et al. investigated populations under three distinct environmental conditions: high humidity and high temperature, low temperature and high humidity, and moderate temperature and low humidity. Results indicate that bacterial populations on the backs, armpits, and feet of individuals were significantly larger in high-humidity, high-temperature environments compared to moderate-temperature, low-humidity conditions. This suggests that high-temperature, high-humidity environmental conditions are conducive to microbial growth and reproduction [7]. Myers T's research found that equine skin microbial diversity was higher in winter and summer than in spring and autumn, though the differences were not statistically significant; microbial community structure clustered more by season than by skin location [8]. These studies have focused on the influence of individual factors on skin microbes, while research on the patterns of skin microbial community change and its underlying mechanisms remains limited. In recent years, an increasing number of studies have focused on the 'gut-skin' axis, which describes the bidirectional signaling occurring between the skin and the gastrointestinal tract under both homeostatic and disease conditions. As a central metabolic organ, the skin exhibits enzymatic activity comparable to that of the gut. Whether metabolites produced by the skin microbiome and associated exogenous enzymes influence intestinal metabolism and systemic homeostasis warrants further investigation [9]. Among these, dietary nutrition is considered one of the most critical environmental factors. As a key factor affecting animal microbial communities, differences in dietary nutrient components may indirectly influence skin microbial composition by altering the host's metabolic state. Research indicates that the ratio of nutrients such as protein, fat, and carbohydrates in the diet significantly affects the gut microbiota of animals [10]. High-protein diets can increase the abundance of proteolytic bacteria and alter the production of microbial metabolites, such as short-chain fatty acids and branched-chain amino acids, which, in turn, affect host immunity and metabolism [11]. Dietary fats, particularly saturated fats, have been linked to reduced microbial diversity and increased intestinal permeability, promoting systemic inflammation that may extend to the skin [12]. Conversely, complex carbohydrates and dietary fibers act as prebiotics, fostering beneficial gut bacteria, enhancing gut barrier integrity, and promoting anti-inflammatory responses. Critically, these diet-driven alterations in gut microbial ecology are transmissible to the skin via the "gut-skin axis," a bidirectional communication network involving immune, metabolic, and neuroendocrine pathways [13]. Through this axis, gut-derived microbial metabolites and inflammatory signals can reach the skin, thereby influencing its microbial colonization, barrier function, and overall homeostasis. This indicates that the 'gut-skin axis' is a crucial pathway through which dietary intake regulates the skin microbiome. However, research on the impact of energy-based diets on the skin microbiota of yaks has largely remained unexplored. This study systematically investigated the effects of the difference in feeding energy levels on the skin microbiota and metabolites of Tianzhu white yak by integrating skin microbiome and metabolomics technologies. The findings will establish a foundation for utilizing skin microbiota as key biomarkers for yak health management and dietary optimization, as well as for research into regulating the gut-skin axis.

2. Materials and Methods

2.1. Animals and Sample Collection

The experiment was conducted in Heimaquanhai Village, Tianzhu County, Gansu Province (altitude: 2800 m). Experimental samples were collected in early May 2023, with a monthly average temperature of 8 to 10 °C and monthly precipitation of 35 to 45 mm (data from Tianzhu County Meteorological Bureau, 2023). Initially, 20 healthy male yaks were selected, including 10 aged 2.5 years (30-month-olds) and 10 aged 4.5 years (54-month-olds). Animals in each age group were randomly divided into two groups and fed with diets of high-energy and low-energy nutritional levels, respectively. All animals were raised in individual pens within a barn, with clean, fresh water and complete mixed feeds with different dietary levels provided *ad libitum*. All yaks were fed twice daily at 9:00 and 16:00 h. Due to the unavoidable loss of one sample from the High-energy feeding group of 2.5-year-old yaks during sampling, the final sample size was 19 yaks. First, yaks from both age groups were randomly assigned to two dietary groups, forming four initial experimental groups, the 2.5-year-old high-nutrit group (H_2.5y, $n = 4$), the 4.5-year-old high-nutrient group (H_4.5y, $n = 5$), the 2.5-year-old low-nutrient group (L_2.5y, $n = 5$), and the 4.5-year-old low-nutrient group (L_4.5y, $n = 5$). (Table 1) For the feeding experiment, the pre-feeding period lasted for 15 days, and the experimental period lasted for 90 days. The experimental diet was formulated as a supplementary feed in accordance with the Chinese Beef Cattle Feeding Standards (NY/T 815—2004) [14]. Based on the nutritional recommendations for beef cattle weighing 150 kg with a daily weight gain of 500 g, complete mixed pelleted feeds were formulated at different dietary levels. The roughage comprised maize straw, oat straw, and astragalus straw, while the concentrate included corn, wheat bran, rapeseed meal, soybean protein powder, salt, and premix. Two diets were formulated: a high-nutrient diet with a concentrate-roughage ratio of 65:35 and a net energy (NE_{mf}) of 97.84 MJ/kg (Group H), and a low-nutrient diet with a concentrate-roughage ratio of 35:65 and a net energy (NE_{mf}) of 81.54 MJ/kg (Group L). The composition and nutritional content of the diet are presented in Table 2. Subsequent β -diversity analysis employing the Bray–Curtis distance revealed a distinct separation between the high-energy and low-energy groups. Consequently, all samples were reclassified into two groups (L and H) to focus subsequent analyses on the core research objective (Table 1). After the feeding period, the yaks were transferred to the slaughter area and humanely slaughtered in accordance with the abattoir's standard operating procedures. Before slaughter, the yaks were restrained and anesthetized via intramuscular injection of xylazine hydrochloride (0.5 mg/kg body weight) and ketamine (5 mg/kg body weight) to minimize animal suffering. During slaughter, yak skin was removed while avoiding cross-contamination. Immediately afterward, sterilized scissors were used to trim and thoroughly clean the scapular hair. Skin microorganisms were collected by repeatedly rubbing the cleaned skin with sterile sampling swabs; a small amount of surrounding hair with roots was plucked, and the hair roots were cut and placed into centrifuge tubes to ensure the collection of more skin-associated microorganisms. A skin tissue sample was collected from an adjacent area. All microbial and skin samples were snap-frozen and stored in liquid nitrogen for subsequent sequencing analysis. All animal experiments, including experimental design and feeding management, were approved by the Animal Ethics Committee of Gansu Agricultural University (Approval No. GSAU-Eth-AST-2023-014).

Table 1. Initial and final experimental grouping.

Initial Group	Age	Initial Sample Size (n)	Dietary Energy Level (NEmf, MJ/kg)	Final Analysis Group	Final Sample Size (n)
H_2.5y	2.5 years	4	97.84 (High)	High-energy group (H)	9
H_4.5y	4.5 years	5	97.84 (High)	High-energy group (H)	9
L_2.5y	2.5 years	5	81.54 (Low)	Low-energy group (L)	10
L_4.5y	4.5 years	5	81.54 (Low)	Low-energy group (L)	10

Table 2. Diet Composition and Nutrient Content Table.

Items	L	H
Diet composition		
Corn straw	38.90	21.00
Oat grass	13.30	7.00
Astragalus straw	12.70	7.00
corn	14.90	27.30
bran	7.20	13.00
rapeseed cake	6.80	13.00
bean protein powder	3.60	6.50
salt	0.60	1.30
gunk	1.90	3.90
Total/	100	100
Nutrient level		
(MJ/kg)	81.54	97.84
Dry matter (DM)	92.97	92.28
Crude protein (CP)	13.43	17.27
Ether extract (EE)	8.95	9.09
Neutral detergent fiber (NDF)	43.42	41.63
Acid detergent fiber (ADF)	21.36	19.40
Ca	0.52	0.71
P	0.34	0.49

2.2. DNA Extraction and PCR Amplification

Genomic DNA from skin microbial samples was extracted using the MagPure Soil DNA LQ Kit (Magan Biotechnology, Guangzhou, China). DNA concentration and purity were assessed using NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. Using barcoded specific primers as templates, the conserved V3-V4 region of the 16S rRNA gene was amplified with forward primer 343F (5'-TACGGRAGGCAGCAG-3') and reverse primer 798R (5'-AGGGTATCTAATCCT-3') [15]. PCR employed Takara's Tks Gflex DNA Polymerase (Takara Bio Inc., Shiga, Japan) in a 30 µL reaction mixture: 15 µL 2× Gflex PCR Buffer, 1 µL Forward Primer (5 pmol/µL), 1 µL Reverse Primer (5 pmol/µL), 1 µL Tks Gflex DNA Polymerase (1.25 U/µL), 0.6 µL Template DNA 50 ng, supplemented with ddH₂O to 30 µL. PCR conditions were as follows: 94 °C pre-denaturation for 5 min; 26 cycles of 94 °C denaturation for 30 s, 56 °C annealing for 30 s, and 72 °C extension for 20 s; followed by a final extension at 72 °C for 5 min, then stored at 4 °C. The PCR products were analyzed by electrophoresis on a 2% agarose gel. Following the analysis, the products were purified using AMPure XP magnetic

beads(Beckman Coulter, Brea, CA, USA), and Qubit quantification was performed. Equal volumes of purified PCR products were pooled and submitted for sequencing.

2.3. Illumina MiSeq Sequencing and Data Analysis

Sequencing libraries were constructed using the Illumina DNA library preparation kit, followed by high-throughput sequencing analysis (Shanghai OE Biotech Co., Ltd., Shanghai, China). Quality control of 16S rRNA raw sequences was performed using FASTQC. After data generation, primer sequences were removed from the raw data using Cutadapt(version 4.4). Subsequently, DADA2 [16] was used to process the validated paired-end raw data from the previous step. This involved quality filtering, denoising, assembly, and de-chimerasing using default parameters in QIIME2 (version 2020.11) [17], yielding representative sequences and an ASV abundance table. These sequences were aligned against the SILVA database (version 138) for species annotation. Species annotation was performed using the q2-feature-classifier plugin within QIIME2, which uses a Naive Bayes classifier by default with a confidence threshold of 0.7. Community bar plots were generated using R. Alpha and beta diversity analyses were conducted using QIIME 2. Chao 1, Shannon, Simpson, and ACE indices were used to assess alpha diversity. The unweighted Unifrac distance matrix, computed in R, was used to perform unweighted Unifrac principal coordinate analysis (PCoA) to assess β -diversity. Differential analysis was performed using the Wilcoxon test in R. LEfSe analysis (LDA > 3.5, $p < 0.05$) was used to identify bacterial taxonomic units. The results of the analyses of the between-group diversity and between-group species difference were corrected for multiple testing using FDR.

2.4. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) Detection

Because skin tissue metabolite detection requires high-integrity sample collection, timely cryopreservation, and high-quality extraction (excessive subcutaneous fat can dilute metabolite concentrations, and residual fascia can introduce interfering metabolites from non-skin tissues), we conducted strict preliminary quality screening of the initial 19 samples. Finally, 12 samples were retained. These 12 samples achieved age-balanced representation within each group (Group H included 3 samples of 2.5 years old and 3 samples of 4.5 years old; Group L also included 3 samples of 2.5 years old and 3 samples of 4.5 years old). Twelve samples (nL = 6, nH = 6) were used for subsequent liquid chromatography-tandem mass spectrometry (LC-MS/MS) non-targeted metabolomics analysis. A total of 50 mg of sample was weighed and mixed with the extraction solution (methanol: water = 4:1). The mixture was then precooled to $-40\text{ }^{\circ}\text{C}$ for 2 min, ground using a homogenizer, and subjected to ultrasonic treatment. Allow the sample to settle for protein precipitation before centrifuging. Transfer the supernatant to a sample vial for LC-MS/MS analysis. The sample is separated using an ultra-high-performance liquid chromatography system (ACQUITY UPLC HSS T3, Waters Corporation, Milford, MA, USA) and detected by mass spectrometry. Mobile phase A consisted of water/acetonitrile (volume ratio 19:1, containing 0.1% formic acid), while mobile phase B consisted of acetonitrile/isopropanol (volume ratio 1:1, containing 0.1% formic acid). Mass spectrometry signals were acquired using ion spray voltage and positive/negative ion scanning modes. To ensure analytical reliability, quality control samples were co-analyzed with the test samples.

2.5. Data Processing and Analysis

The raw data were converted into the. mzXML format by ProteoWizard (version 3.0.2), and then the XCMS program (version 3.16.0) was used for peak alignment, Retention time correction, and extraction of peak areas. Compounds were identified using the Human Metabolome Database (HMDB), Lipidmaps (V2.3), Metlin, and a self-built database based on accurate mass-to-charge ratios (M/z), secondary fragments, and isotopic distributions.

For the data obtained from XCMS extraction, ion peaks with >50% missing values within the group were removed. The software SIMCA-P 14.1 (Umetrics, Umea, Sweden) was used for pattern recognition. After Pareto-scaling preprocessing, multi-dimensional statistical analysis was performed, including PCA, PLS-DA, and OPLS-DA. When evaluating the quality of the established models, three important indicators, namely R2X, R2Y, and Q2, are mainly referred to. Among them, R2X is used to quantify the model's optimization and reflects the total variation in the X variables. At the same time, R2Y reports the percentage of variation in the response variable Y, reflecting the model's explanatory power for Y. Unidimensional statistical analyses included Student's *t*-test and fold change analysis, and R software was used to plot the volcano plot. Based on variable importance in projection (VIP) values from the OPLS-DA model and *p*-values from Student's *t*-tests, after FDR correction, the significantly different metabolites were identified. Metabolites with VIP > 1 and *p* < 0.05 were considered differential metabolites. Pathways for differential metabolites were obtained by metabolic pathway annotation in the KEGG database. Correlation analysis was conducted between differential skin tissue metabolites and differential microbiota using R4.2.3 to assess Spearman's correlation between the differential yak skin microbiota and differential skin tissue metabolites. This analysis aimed to explore the correlative relationships between skin microorganisms and their metabolites, with the results visualized as a heatmap. The correlation results were also corrected using FDR to control the false positive rate in multiple testing.

3. Results

3.1. Skin Microbial Diversity and Abundance

After quality control of raw data from 19 samples, the final usable data for analysis ranged from 52,350 to 68,617. A total of 8535 ASVs were identified, with individual sample counts ranging from 506 to 1384. The L_4.5y group yielded the highest number of detected ASVs, averaging 1258, whereas the H_2.5y group yielded the lowest, averaging 756. A total of 76 ASVs were common across all 19 samples (Figure 1A). Annotation of samples across taxonomic levels revealed that all detected skin microbial bacteria were classified into 27 phyla, 57 classes, 149 orders, 273 families, and 622 genera. According to the ASVs' evolutionary tree. (Figure 1B) The high-energy group (H_4.5y and H_2.5y) exhibited similar ASV abundance patterns; for example, the Proteobacteria phylum showed a higher abundance in the high-energy group than in the low-energy group. The figure shows the top 15 bacterial taxa ranked by average abundance at the phylum and genus levels across all samples. The results indicate that Firmicutes, Actinobacteriota, Proteobacteria, and Bacteroidota exhibited the highest relative abundances, with these four phyla collectively accounting for 99.3% of total relative abundance (Figure 1C). At the genus level, highly abundant taxa included *Atopostipes* (12.6%), *Corynebacterium* (10.4%), *Psychrobacter* (10%), *Jeotgalicoccus* (4.8%), and *Romboutsia* (2.9%) (Figure 1D).

3.2. Analysis of α -Diversity and β -Diversity of Skin Microbiota in Yak Populations Maintained at High and Low Nutritional Levels

Alpha diversity indices reflect the richness and diversity of skin microbiota communities. ACE, Chao1, Shannon, and Simpson indices were calculated for each sample (Figure 2A). The results indicate that both age groups in the low-energy diet group had higher alpha indices than those in the high-energy diet group. The 4.5-year-old low-energy group (L_4.5y) demonstrated significantly higher ACE, Chao1, and Shannon indices than the high-energy group (H_4.5y) (*p* < 0.05). The 2.5-year-old low-energy group also exhibited a considerably higher Simpson index than the high-energy group (*p* < 0.05). Under identical nutritional conditions, the α -diversity index of the skin microbiota in 4.5-year-old

yaks exceeded that of 2.5-year-old yaks. The Good's coverage of each sample exceeded 99, indicating comprehensive sequencing coverage of each bacterial community. Principal coordinate analysis (PCoA) based on Bray–Curtis distances revealed that PC1 and PC2 explained 26.9% and 9.64% of community variance, respectively (Figure 2B). This indicates substantial differences in skin microbial communities between the distinct energy feeding groups, with pronounced inter-group variation. PERMANOVA analysis further confirmed that dietary energy level exerted a highly significant effect on yak skin microbial community structure (pseudo- $F = 5.38$, $R^2 = 0.241$, permutations = 999, $p = 0.001$), with this grouping variable explaining 24.1% of the variation in community β -diversity. All samples were subsequently analyzed as high- or low-energy groups, with the primary focus on investigating the impact of nutritional level variation on skin microbial differences. First, analysis of alpha indices revealed that all measured alpha indices were significantly higher in the low-energy group than in the high-energy group ($p < 0.05$) (Table 3).

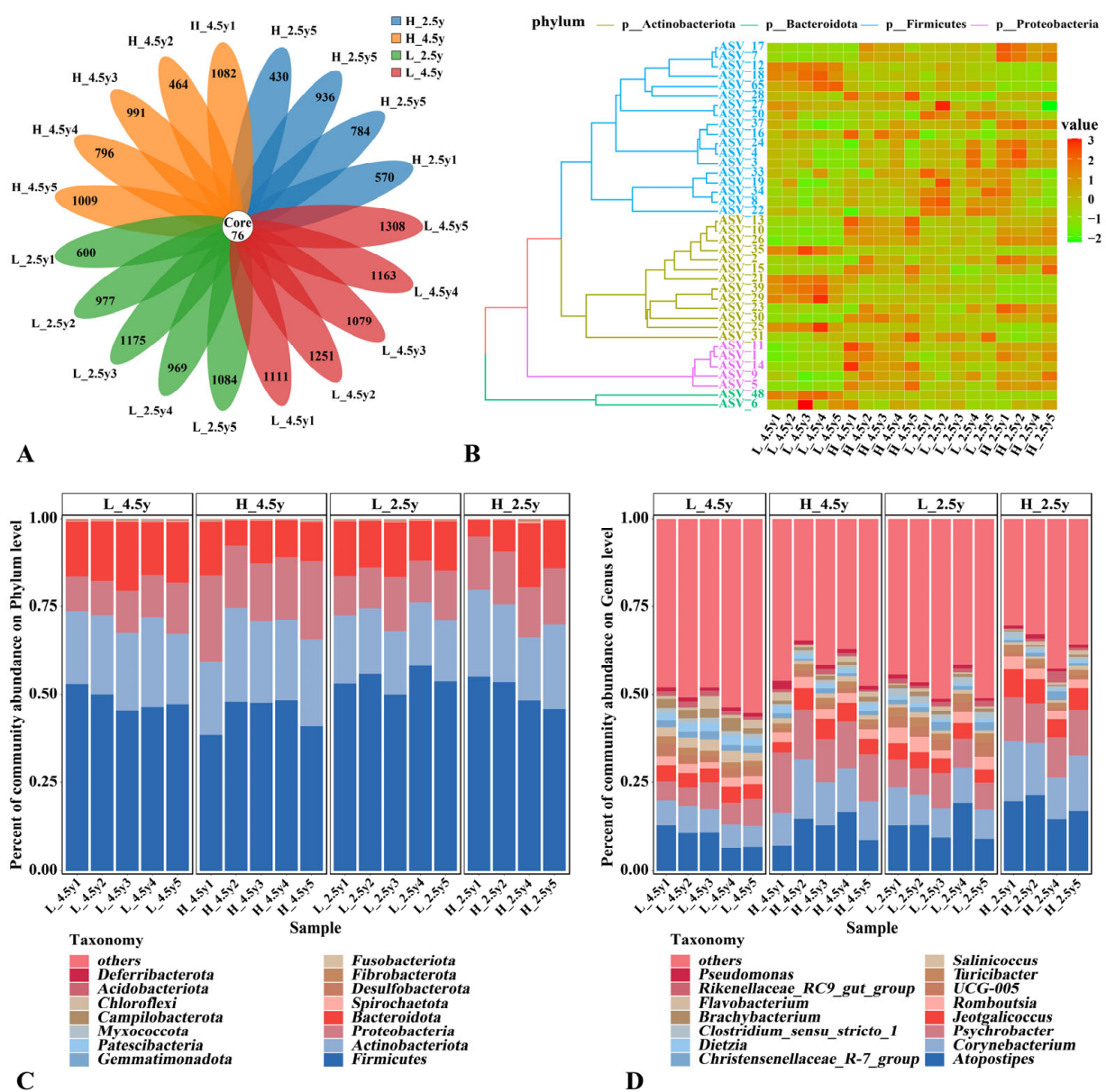


Figure 1. Skin microbial diversity and abundance maps. (A) Venn diagram of skin flora diversity; (B) Species Phylogenetic Tree and ASV Abundance Plot Community compositions of yak skin bacteria; (C) Distribution at the phylum level; (D) Distribution at the genus level. Note: ASV = Amplicon Sequence Variant.

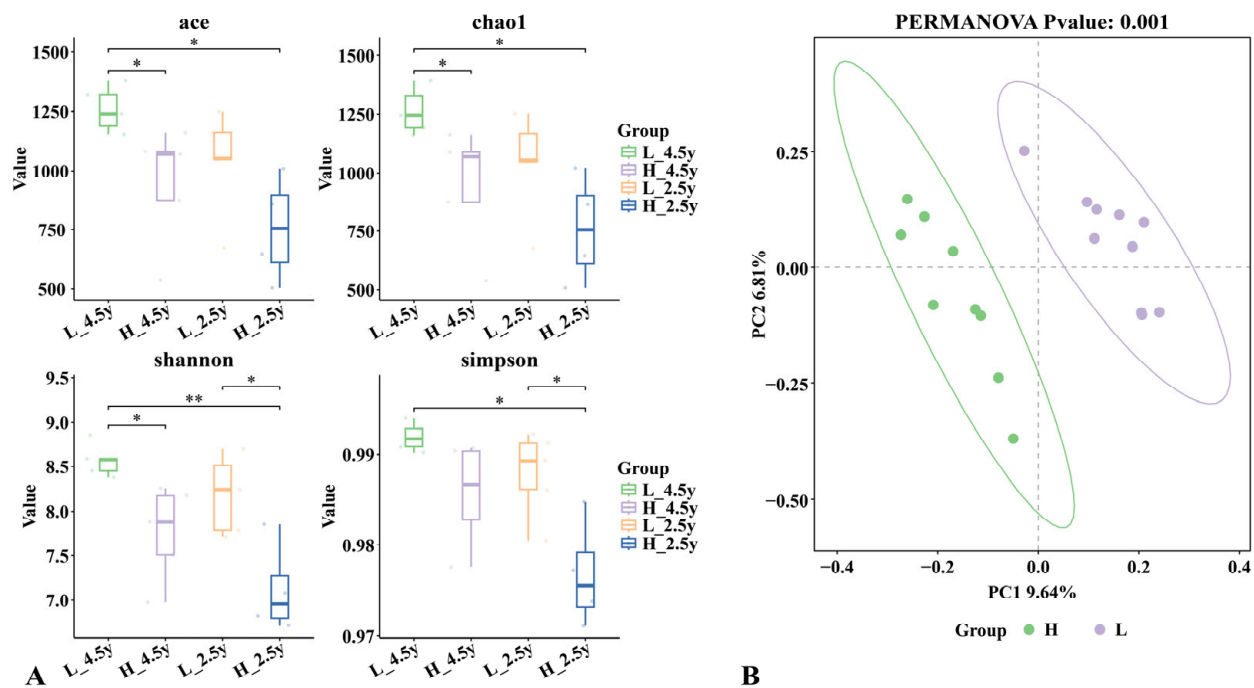


Figure 2. Analysis of α Diversity and β Diversity in Skin Microbiota. (A) Analysis of alpha diversity; (B) PCoA analysis. * indicates $p < 0.05$, ** indicates $p < 0.01$ in the boxplots (A), green represents the L_4.5y group, orange represents the H_4.5y group, purple represents the L_2.5y group, and blue represents the H_2.5y group. In the PCoA plot (B), green represents group H and purple represents group L.

Table 3. α diversity index analysis of different energy levels.

Item	Shannon	Simpson	ACE	Chao1
H	7.47 ± 0.2^b	0.982 ± 0.002^b	854.01 ± 80.19^b	863 ± 81.88^b
L	8.38 ± 0.12^a	0.990 ± 0.001^a	1139.79 ± 61.58^a	1151.21 ± 62.74^a

Note: ^{a,b} in the same column of the above table indicate significant differences ($p < 0.05$), while the same letters indicate no significant differences ($p > 0.05$). Data are pooled from 2.5-year-old and 4.5-year-old yaks in each energy group.

3.3. Differences in the Relative Abundance of Skin Bacteria

Differential analysis of the two sample groups using the Wilcoxon algorithm identified 339 differentially abundant ASVs, 123 differentially abundant genera, and 5 differentially abundant phyla. Among the differentially abundant phyla, the Bacteroidota, Spirochaetota, and Gemmatimonadota phyla were significantly higher in Group L than in Group H ($p < 0.05$). In comparison, the Actinobacteriota and Proteobacteria phyla were significantly higher in Group H than in Group L ($p < 0.05$) (Figure 3A). Among the top ten differentially abundant genera, *Rikenellaceae_RC9_gut_group*, *Christensenellaceae_R-7_group*, *Brachybacterium*, *Chryseobacterium*, *Clostridium_sensu_stricto_1*, *Eubacterium_coprostanoligenes_group*, and *UCG-005* were significantly higher in Group H than in Group L ($p < 0.05$). At the same time, the abundances of *Jeotgalicoccus*, *Corynebacterium*, and *Psychrobacter* in Group H were significantly higher than in Group L ($p < 0.05$) (Figure 3B).

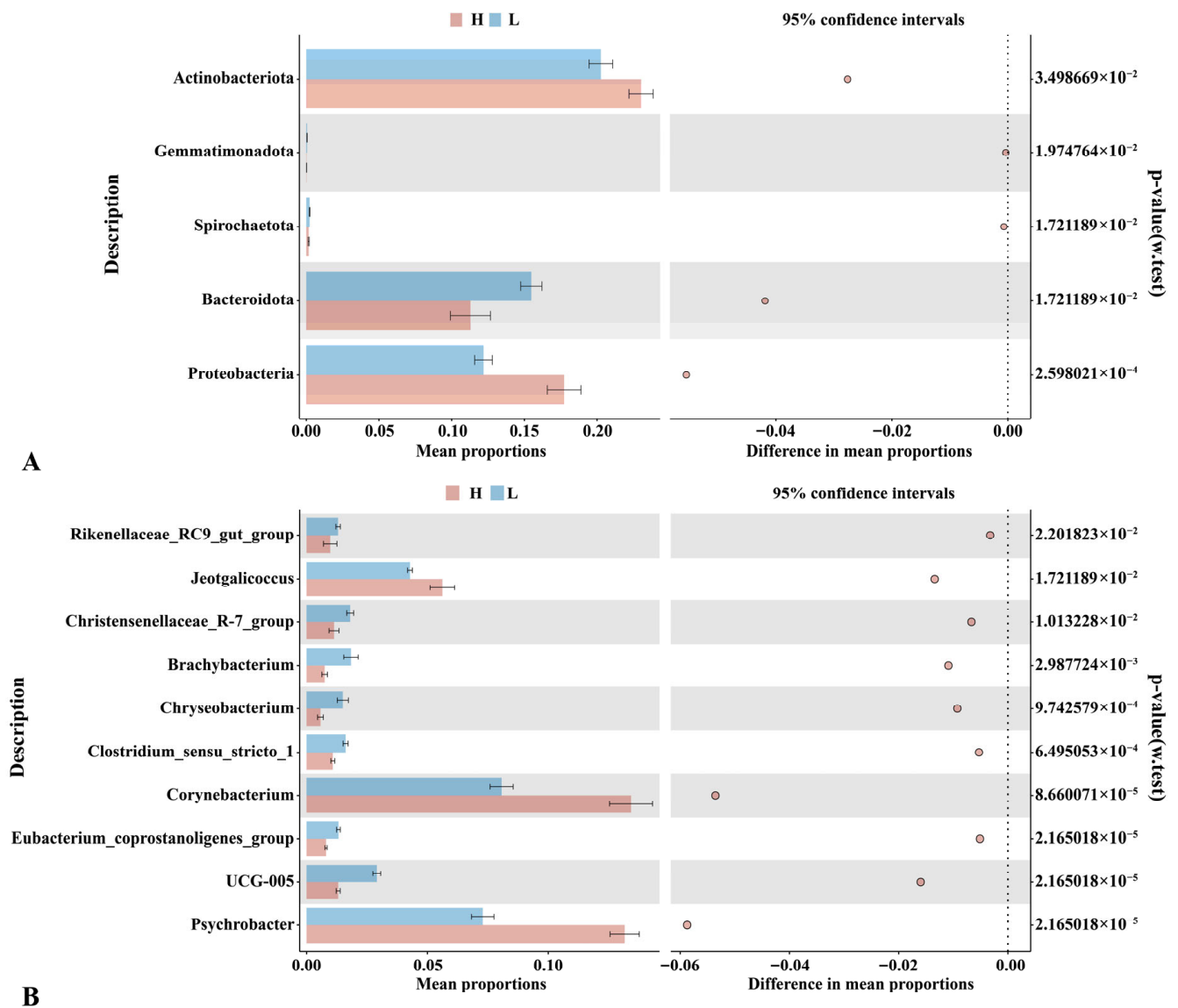


Figure 3. Plot of differences in relative abundance of skin bacteria. (A) analysis of differences in flora at the level of phylum; (B) Analysis of differences in flora at the level of genus.

3.4. LEfSe Analysis of Skin Microbiota

We employed linear discriminant analysis effect size (LEfSe) for multilevel species-difference analysis, comparing microbial abundances across groups to identify differentially abundant species. Results (Figure 4) indicate that 3 and 7 biomarkers were identified in the high-energy and low-energy feeding groups, respectively. The high-energy feeding group was primarily characterized by three biomarkers: *Psychrobacter*, *Corynebacterium*, and *Jeotgalicoccus*. Microorganisms, including *UCG_005*, *Chryseobacterium*, and *Brachybacterium*, dominated the low-energy feeding group.

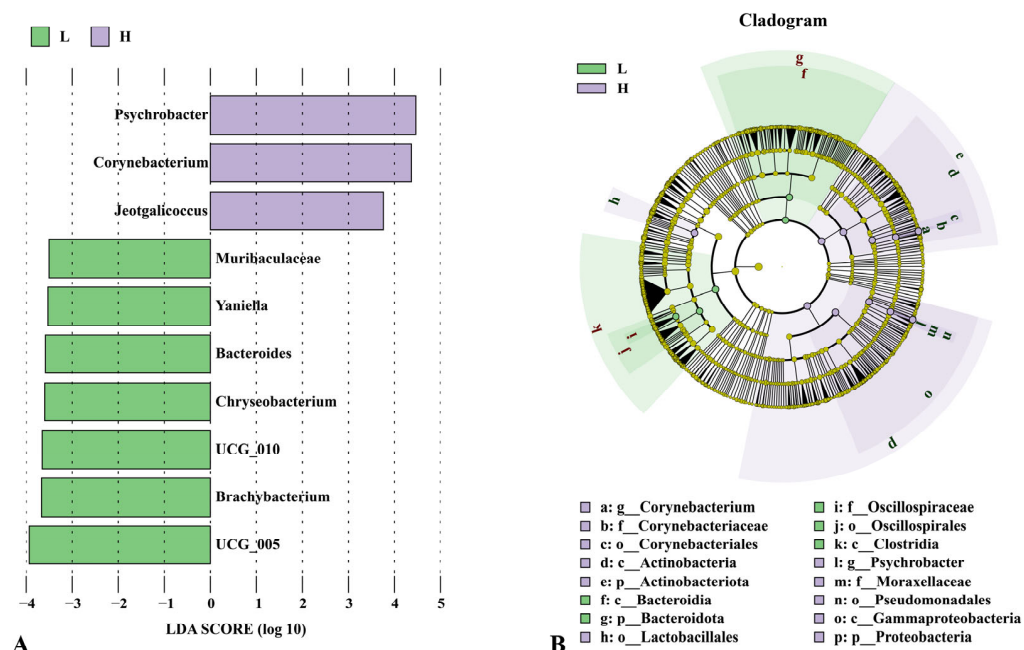


Figure 4. Linear Discriminant Analysis (LDA) effect size (LEfSe) analysis of the skin of Tianzhu white yaks across different energy groups. The LEfSe analysis histogram for the different energy-feeding groups (A). The ordinate represents the taxonomic groups that are significantly different from each other, and the bar chart shows the LDA log scores of each taxonomic group. The longer the bar, the greater the difference between the taxonomic groups. (B) LEfSe analysis of each segment of the gastrointestinal tract. The size of the nodes corresponds to the average relative abundance of the taxonomic group, and the hollow nodes indicate taxonomic groups with no significant difference between groups. These letters identify the names of taxonomic groups that differ greatly.

3.5. Metabolite Analysis of Tissue Differences in Yak Skin

From the Principal Component Analysis (PCA) score plot (Figure 5A), the metabolic components of the samples in the L group and the H group show a partial separation trend, but they are not completely separated. Using Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) (Figure 5B), significant separation and good aggregation were observed. To assess overfitting, 7-fold cross-validation and 200 repetitions of Response Permutation Testing (RPT) were used to evaluate the model's performance. A permutation test statistically validated the OPLS-DA model, yielding $R^2X = 0.579$, $R^2Y = 0.99$, and $Q^2 = 0.356$. R^2Y is close to 1, indicating that the model is not overfitting. The greater the separation between the two groups, the more significant the classification effect. There was a substantial difference between the L group and the H group ($p < 0.05$).

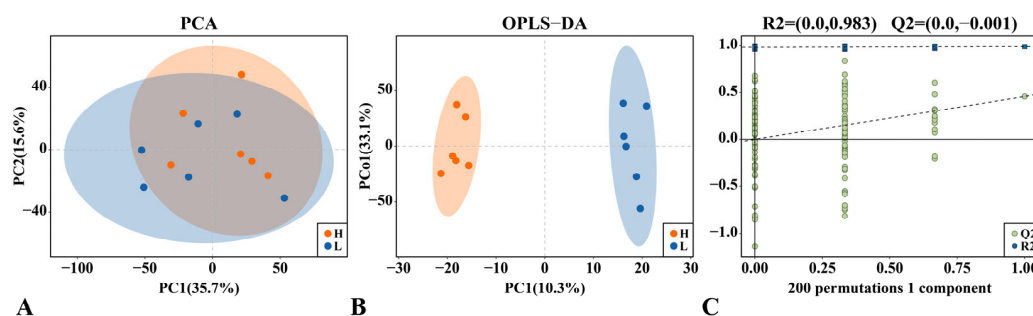


Figure 5. Multivariate statistical analysis of skin tissue metabolome; (A) Plot of PCA scores; (B) OPLS-DA score chart; (C) permutation graph. Note: PCA = principal component analysis; OPLS-DA = orthogonal partial least squares discriminant analysis.

3.6. Differential Metabolite Screening and KEGG Pathway Enrichment Analysis

To confirm significant differences in skin tissue metabolites among Tianzhu white yaks across nutritional levels, 114 metabolites were identified as significantly different ($FC \geq 1.2$, $p < 0.05$). Of these, 73 were upregulated and 41 were downregulated. The results were visualized using a volcano plot (Figure 6A). Using the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway prediction, we analyzed functional differences in genes and genomes. Bacteria from different energy feeding groups exhibited significant functional disparities at the secondary level of KEGG metabolic pathways. KEGG pathway enrichment analysis (Figure 6B) revealed that the top 5 signaling pathways significantly enriched with differential metabolites were: Choline metabolism in cancer, Synaptic vesicle cycle, glycerophospholipid metabolism, arachidonic acid metabolism, and cocaine addiction. Among these, differential metabolites exerted the most pronounced effect on the choline metabolism pathway in cancer.

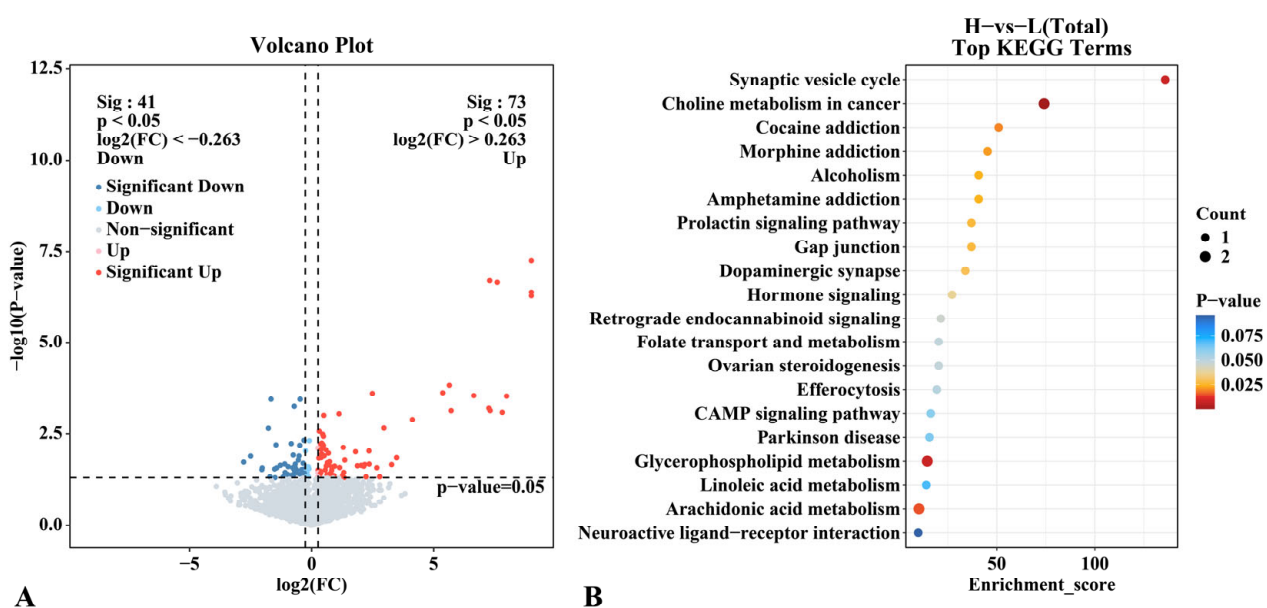


Figure 6. Differential metabolite screening and KEGG pathway enrichment analysis. (A) Differential metabolite volcano map; (B) Differential Metabolite Pathways.

3.7. Correlation Analysis of Skin Microbiota and Tissue-Specific Metabolites

To further explore the relationship between differentially abundant microbial taxa (at the genus level) and metabolites identified in microbial diversity and metabolomic analyses, a heatmap was generated from correlation data between the top 20 VIP-scored differentially abundant metabolites and microbes from both groups (Figure 7). The genus *Psychrobacter* showed a significant positive correlation with stress metabolites, such as corticosterone, suggesting its involvement in skin stress-immune regulation under frigid conditions. Lipid metabolism-related genera, such as *Corynebacterium*, were closely associated with skin barrier metabolites, including palmitic acid, suggesting their role in sebum degradation and in maintaining an acidic microenvironment. Additionally, negative correlations between specific microbial genera and antimicrobial metabolites indicated that microbial communities compete for metabolites to regulate microbiome homeostasis. These associative networks confirm that dietary nutrition modulates skin microbial composition, thereby controlling its metabolic functions and ultimately influencing the host's local immunity, barrier integrity, and adaptability to high-altitude cold environments.

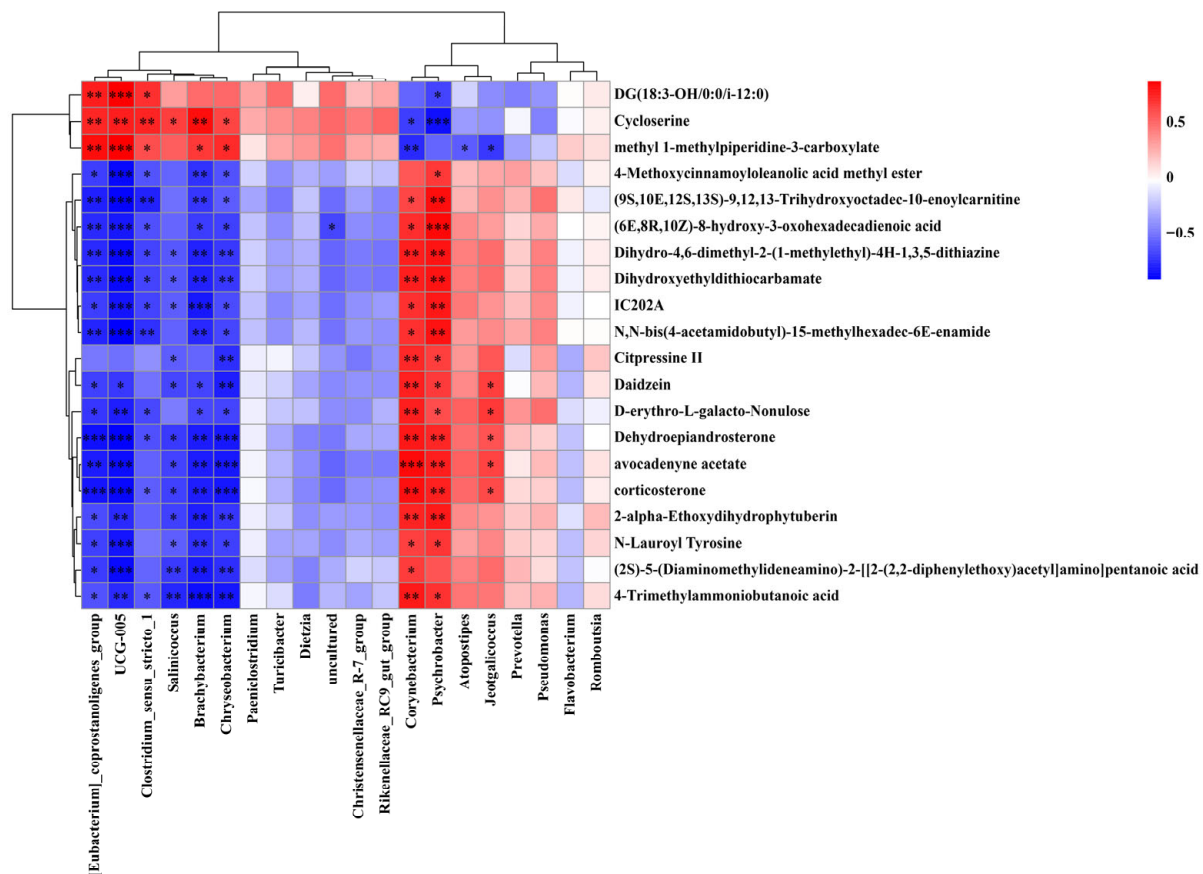


Figure 7. Correlation of skin microorganisms and differential metabolites of skin tissue. * $0.01 < p \leq 0.05$, ** $0.001 < p \leq 0.01$, and *** $p \leq 0.001$.

4. Discussion

This study systematically investigated how dietary energy levels regulate skin microbiota and skin tissue in Tianzhu white yak, revealing that dietary energy significantly modulates skin microbial diversity, community composition, and metabolic pathways. These findings provide new insights into the interactions between yak nutrition and the skin microbiome, with significant theoretical and practical implications. Analysis of the skin bacterial communities of the Tianzhu white yak revealed differences in their composition and structure. Our findings indicate that skin microbial diversity (measured by the Shannon, Simpson, ACE, and Chao1 indices) was significantly higher in the low-energy group than in the high-energy group. This aligns closely with studies of ruminant gut microbiota [18], where higher roughage content in low-energy diets likely provides more complex substrates, creating diverse ecological niches for skin microbes [19]. In contrast, high-energy diets (with a high proportion of concentrate feed) provide concentrated nutrients, favoring the proliferation of specific dominant groups and thereby reducing overall diversity [20]. Furthermore, PCoA analysis revealed a distinct separation in skin microbial community composition between the high- and low-energy groups, indicating that dietary energy significantly influences skin microbial community structure. The pronounced clustering observed in PCoA confirms that energy intake level is the primary driver of community structure.

The four dominant phyla (Firmicutes, Actinobacteriota, Proteobacteria, and Bacteroidota) accounted for 99.3% of total abundance, underscoring their pivotal role in maintaining the skin microbiome's equilibrium. Firmicutes enriched in the low-energy group secrete lipophosphate and trace amines that suppress skin inflammation and pro-

mote wound healing [21]. The high crude fiber content in low-energy diets may provide abundant cellulose for Firmicutes, thereby enhancing their role in lipid metabolism and in maintaining barrier function [20]. Actinobacteriota, enriched in the high-energy group, dominate sebum-rich skin sites and participate in cholesterol metabolism and ceramide synthesis [22]. High-energy diets increase skin lipid content, providing additional metabolic substrates for Actinobacteriota and enhancing their antimicrobial and barrier-protective functions [20]. Notably, the Firmicutes/Actinobacteriota (F/A) ratio, a marker of skin health, differed significantly between groups [23], indicating that dietary energy can influence skin health by modulating this ratio. This parallels the association between the Firmicutes/Bacteroidota (F/B) ratio and fat deposition observed in ruminants [24].

LEfSe-identified biomarkers further highlight microbial adaptive traits in response to dietary conditions. The cold-tolerant genus *Psychrobacter*, enriched in the high-energy group, positively correlates with corticosterone levels [25], suggesting it may help yaks counteract metabolic stress from high-energy diets while adapting to cold environments [26]. *Corynebacterium*, another dominant genus in the high-energy group, is a common skin commensal widely distributed across human and animal cutaneous surfaces, particularly in humid regions such as the axillary folds and interdigital spaces [27]. Its association with palmitic acid suggests a role in sebum degradation and in maintaining the skin's acidic microenvironment [28]. Within the low-energy group, *UCG-005* (a member of the *Ruminococcus* family) regulates energy balance by producing short-chain fatty acids [29]. In Li's study of dairy cattle, *UCG_005* abundance was significantly correlated with enhanced activity of starch and sucrose metabolic pathways [30]. Furthermore, *UCG_005* is closely associated with lactic acid synthesis, which may indirectly improve skin health by influencing the skin's lipid barrier function [31]. Meanwhile, the genus *Xanthomonas* enhances skin antioxidant capacity and immune regulation [32], thereby compensating for potential nutritional limitations.

LC-MS/MS analysis of skin tissue from Tianzhu white yaks revealed 114 differentially abundant metabolites between high- and low-energy feeding groups. These metabolites were enriched in lipid metabolism (glycerophospholipid metabolism), signal transduction (synaptic vesicle recycling), and stress response (choline metabolism in cancer) pathways, underscoring the comprehensive regulatory role of dietary energy in skin metabolism. Glycerophospholipid metabolism, a key pathway for cell membrane synthesis and signal transduction, was significantly enriched, indicating that dietary energy influences skin barrier integrity by regulating lipid metabolism. Choline metabolism, closely linked to keratinocyte proliferation and differentiation, was also markedly enriched, suggesting that high-energy diets may enhance epidermal barrier function via choline-derived metabolites such as phosphatidylcholine [33]. Furthermore, synaptic vesicle recycling was significantly enriched in both groups; it is involved in neurotransmitter release at nerve terminals, thereby regulating skin perception and immune responses [34]. Regardless of energy levels, skin microbiota helps yaks cope with cold stress by mediating neuro-immune interactions [35]. Metabolic differences between groups reflect adaptive strategies: the high-energy diet upregulates choline, amino acid, and fatty acid metabolites to support heightened metabolic activity, whereas the low-energy diet increases organic acid and carbohydrate metabolites to maintain basal metabolic equilibrium [36]. These findings align with Yang et al.'s [37] investigation of bovine lipid metabolism, confirming a consistent pattern of dietary energy level regulation across ruminant metabolic pathways.

By combining analysis of skin microbial genera and differential metabolites, it was found that associations between microorganisms and metabolites are highly specific and regular. First, *Psychrobacter* and *Corynebacterium*, both enriched in the high-energy group, showed strong positive correlations with corticosterone. Corticosterone is a key gluco-

corticoid hormone that plays a crucial role in the body's stress response. Elevated levels suggest that high-energy diets, while providing adequate nutrition, may also impose specific metabolic stress on yaks [38]. *Psychrobacter*, a psychrophilic and lipophilic bacterium, may not only adapt to high-fat environments but also utilize or respond to corticosterone, thereby occupying a dominant ecological niche within this microenvironment. Genera associated with the low-energy group exhibit distinct patterns of metabolite association. Notably, bacterial genera such as *UCG-005*, which are more closely associated with the L-group community structure, show negative correlations with metabolites, including 4-Trimethylammoniobutanoic acid (endogenous carnitine). Carnitine plays a pivotal role in fatty acid β -oxidation, and its reduced levels may reflect a general slowing of energy metabolism in skin tissue under low-energy diets [37]. Furthermore, specific L-group-associated bacterial genera with fiber-degrading potential showed positive correlations with short-chain fatty acid (SCFA) precursor metabolites. This suggests that such microbial communities may generate SCFAs through fermentation, thereby contributing to the maintenance of the skin barrier's acidic environment and immune homeostasis [39]. This is consistent with the higher microbial diversity observed in the low-energy feeding group.

5. Conclusions

This study shows that diets with different energy levels significantly affect the composition of the skin microbial community and metabolites in Tianzhu white yaks. A low-energy diet promotes diversity within the skin microbial community, whereas a high-energy diet increases the abundance of specific microbial groups, particularly those associated with skin barrier function and immune protection. The findings provide new insights into how diet regulates the skin microbial community and its health, and are particularly significant for the breeding and management of yaks in cold regions. Future research should increase sample size and examine different environmental conditions to verify the universality of these findings.

Author Contributions: Author Contributions: P.Z. and B.S.: Data analysis, writing, X.Z. (Xuelan Zhou) and Z.Z.: Article revision and review. X.Z. (Xiaolan Zhang) and J.H.: Project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Natural Science Foundation of China (NSFC), grant number 32302720; the Gansu Agricultural University Public Recruitment Doctoral Research Start-up Fund (GAU-KYQD-2022-18).

Institutional Review Board Statement: This study involving animals was approved by the Animal Ethics Committee of Gansu Agricultural University (approval number GSAU-Eth-AST-2023-014, approval date on 2023-03-09).

Informed Consent Statement: Not applicable.

Data Availability Statement: The raw 16S rRNA sequencing data (FASTQ format) and sample metadata have been deposited in the NCBI Sequence Read Archive (SRA) database under the BioProject accession number PRJNA1416637.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. De, J.; Sun, K.X.; Luo, Q. Research progress on physiological characteristics and plateau adaptive molecular genetics of Tibetan sheep and yaks. *J. Domest. Anim. Ecol.* **2022**, *43*, 1–7.
2. Geng, Y. Study on the Structure and Function of Yak Skin. Master's Thesis, Lanzhou University, Lanzhou, China, 2006.
3. Zimmerman, A.; Bai, L.; Ginty, D.D. The gentle touch receptors of mammalian skin. *Science* **2014**, *346*, 950–954. [[CrossRef](#)]

4. Yang, X.; Cui, Y.; Yue, J.; He, H.; Yu, C.; Liu, P.; Liu, J.; Ren, X.; Meng, Y. The histological characteristics, age-related thickness change of skin, and expression of the HSPs in the skin during hair cycle in yak (*Bos grunniens*). *PLoS ONE* **2017**, *12*, e0176451. [\[CrossRef\]](#)
5. Ruan, C.M.; Wang, J.; Yang, Y.X.; Hu, J.J.; Ma, Y.J.; Zhang, Y.; Zhao, X.X. Proteomic analysis of Tianzhu White Yak (*Bos grunniens*) testis at different sexual developmental stages. *Anim. Sci. J.* **2019**, *90*, 333–343. [\[CrossRef\]](#)
6. Park, K. Role of micronutrients in skin health and function. *Biomol. Ther.* **2015**, *23*, 207–217. [\[CrossRef\]](#) [\[PubMed\]](#)
7. McBride, M.E.; Duncan, W.C.; Knox, J.M. The environment and the microbial ecology of human skin. *Appl. Environ. Microbiol.* **1977**, *33*, 603–608. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Myers, T.; Bouslimani, A.; Huang, S.; Hansen, S.T.; Clavaud, C.; Azouaoui, A.; Ott, A.; Gueniche, A.; Bouez, C.; Zheng, Q.; et al. A multi-study analysis enables identification of potential microbial features associated with skin aging signs. *Front. Aging* **2024**, *4*, 1304705. [\[CrossRef\]](#)
9. Wood, J.M.; Schallreuter, K.U. A plaidoyer for cutaneous enzymology: Our view of some important unanswered questions on the contributions of selected key enzymes to epidermal homeostasis. *Exp. Dermatol.* **2008**, *17*, 569–578. [\[CrossRef\]](#)
10. Wang, Y.; Shi, M.; Wu, J.; Han, X.; Li, M.; Wu, Y.; Jiang, Y.; Zhang, H.; Liu, S.; Hu, D. Variations in Intestinal Microbiota Among Three Species in the Cervidae Family Under the Same Feeding Conditions. *Vet. Sci.* **2025**, *12*, 438. [\[CrossRef\]](#)
11. Smith, R.P.; Easson, C.; Lyle, S.M.; Kapoor, R.; Donnelly, C.P.; Davidson, E.J.; Parikh, E.; Lopez, J.V.; Tartar, J.L. Gut microbiome diversity is associated with sleep physiology in humans. *PLoS ONE* **2019**, *14*, e0222394. [\[CrossRef\]](#) [\[PubMed\]](#)
12. De Pessemier, B.; Grine, L.; Debaere, M.; Maes, A.; Paetzold, B.; Callewaert, C. Gut-Skin Axis: Current Knowledge of the Interrelationship between Microbial Dysbiosis and Skin Conditions. *Microorganisms* **2021**, *9*, 353. [\[CrossRef\]](#)
13. Salem, I.; Ramser, A.; Isham, N.; Ghannoum, M.A. The Gut Microbiome as a Major Regulator of the Gut-Skin Axis. *Front. Microbiol.* **2018**, *9*, 1459. [\[CrossRef\]](#)
14. NY/T 815-2004; Agricultural Industry Standard of the People's Republic of China—Feeding Standard of Beef Cattle. Hunan Feed 05. Ministry of Agriculture of the People's Republic of China: Beijing, China, 2004; pp. 14–18.
15. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berg-Lyons, D.; Lozupone, C.A.; Turnbaugh, P.J.; Fierer, N.; Knight, R. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 4516–4522. [\[CrossRef\]](#) [\[PubMed\]](#)
16. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [\[CrossRef\]](#)
17. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [\[CrossRef\]](#)
18. Clarke, S.F.; Murphy, E.F.; O'Sullivan, O.; Lucey, A.J.; Humphreys, M.; Hogan, A.; Hayes, P.; O'Reilly, M.; Jeffery, I.B.; Wood-Martin, R.; et al. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* **2014**, *63*, 1913–1920. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Patel, V.; Patel, A.K.; Parmar, N.R.; Patel, A.B.; Reddy, B.; Joshi, C.G. Characterization of the rumen microbiome of Indian Kankrej cattle (*Bos indicus*) adapted to different forage diet. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 9749–9761. [\[CrossRef\]](#)
20. Sun, Y.; Zhang, S.; Nie, Q.; He, H.; Tan, H.; Geng, F.; Ji, H.; Hu, J.; Nie, S. Gut firmicutes: Relationship with dietary fiber and role in host homeostasis. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 12073–12088. [\[CrossRef\]](#)
21. Park, J.Y.; Lee, J.Y.; Hong, S.; Heo, H.; Lee, H.; Kim, Y.G.; Kim, B.K.; Choi, S.I.; Lee, J. *Limosilactobacillus fermentum* MG5368 and *Lactiplantibacillus plantarum* MG989 Regulates Skin Health in UVB-Induced HaCaT Cells and Hairless Mice Model. *Nutrients* **2024**, *16*, 4083. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Fusco, A.; Perfetto, B.; Savio, V.; Chiaromonte, A.; Torelli, G.; Donnarumma, G.; Baroni, A. Regulatory Ability of *Lactiplantibacillus plantarum* on Human Skin Health by Counteracting In Vitro *Malassezia furfur* Effects. *J. Fungi* **2023**, *9*, 1153. [\[CrossRef\]](#)
23. Grice, E.A.; Segre, J.A. The skin microbiome. *Nat. Rev. Microbiol.* **2011**, *9*, 244–253. [\[CrossRef\]](#)
24. Ley, R.E.; Turnbaugh, P.J.; Klein, S.; Gordon, J.I. Microbial ecology: Human gut microbes associated with obesity. *Nature* **2006**, *444*, 1022–1023. [\[CrossRef\]](#)
25. Ioannou, P.; Ziogou, A.; Giannakodimos, A.; Giannakodimos, I.; Tsantes, A.G.; Samonis, G. *Psychrobacter* Infections in Humans-A Narrative Review of Reported Cases. *Antibiotics* **2025**, *14*, 140. [\[CrossRef\]](#)
26. Hurtado-Ortiz, R.; Nazimoudine, A.; Criscuolo, A.; Hugon, P.; Mornico, D.; Brisse, S.; Bizet, C.; Clermont, D. *Psychrobacter pasteurii* and *Psychrobacter piechaudii* sp. nov., two novel species within the genus *Psychrobacter*. *Int. J. Syst. Evol. Microbiol.* **2017**, *67*, 3192–3197. [\[CrossRef\]](#)
27. Ahmed, N.; Joglekar, P.; Deming, C.; NISC Comparative Sequencing Program; Lemon, K.P.; Kong, H.H.; Segre, J.A.; Conlan, S. Genomic characterization of the *C. tuberculostearicum* species complex, a prominent member of the human skin microbiome. *mSystems* **2023**, *8*, e0063223. [\[CrossRef\]](#)

28. Kaur, J.; Sood, U.; Talwar, C.; Whitman, W.B.; Lal, R. Phylogenomics-based reclassifications in the genus *Psychrobacter* including emended descriptions of *Psychrobacter pacificensis*, *Psychrobacter proteolyticus* and *Psychrobacter submarinus*. *Antonie Van Leeuwenhoek* **2023**, *116*, 1113–1121. [[CrossRef](#)]
29. Huang, W.; Wang, J.; Xiao, Z.; Lin, J.; Tan, Z.; Sun, G. Lingguizhugan decoction alleviates obesity in rats on a high-fat diet through the regulation of lipid metabolism and intestinal microbiota. *Front. Microbiol.* **2024**, *15*, 1462173. [[CrossRef](#)]
30. Li, Y.; Lv, M.; Wang, J.; Tian, Z.; Yu, B.; Wang, B.; Liu, J.; Liu, H. Dandelion (*Taraxacum mongolicum* Hand.-Mazz.) Supplementation-Enhanced Rumen Fermentation through the Interaction between Ruminal Microbiome and Metabolome. *Microorganisms* **2020**, *9*, 83. [[CrossRef](#)]
31. Zhao, Y.; Guo, Y.; Yang, C.; Song, Z.; Luo, X. Differences in Milk Fatty Acids Profile of Two Breeds of Water Buffaloes Explained by Their Gastrointestinal Microbiota. *Animals* **2024**, *14*, 2146. [[CrossRef](#)]
32. Wen, J.; He, J.Q. The Causal Impact of the Gut Microbiota on Respiratory Tuberculosis Susceptibility. *Infect. Dis. Ther.* **2023**, *12*, 2535–2544. [[CrossRef](#)]
33. Wu, X.; Zhang, G.; Zhang, W.; Zhou, J.; Cong, H.; Yang, G.; Liu, G. Rumen microbiota helps Tibetan sheep obtain energy more efficiently to survive in the extreme environment of the Qinghai-Tibet Plateau. *Front. Microbiol.* **2024**, *15*, 1431063. [[CrossRef](#)] [[PubMed](#)]
34. Wang, S.; Kong, F.; Liu, J.; Xia, J.; Du, W.; Li, S.; Wang, W. Comparative Analysis of Rumen Microbiota Composition in Dairy Cows with Simple Indigestion and Healthy Cows. *Microorganisms* **2023**, *11*, 2673. [[CrossRef](#)] [[PubMed](#)]
35. Belkaid, Y.; Tamoutounour, S. The influence of skin microorganisms on cutaneous immunity. *Nat. Rev. Immunol.* **2016**, *16*, 353–366. [[CrossRef](#)]
36. Chen, Y.E.; Fischbach, M.A.; Belkaid, Y. Skin microbiota-host interactions. *Nature* **2018**, *553*, 427–436. [[CrossRef](#)]
37. Yang, C. Research on the Molecular Mechanism of Cattle Fat Metabolism in Response to Dietary Energy Levels. Master's Thesis, Lanzhou University, Lanzhou, China, 2018. (In Chinese)
38. Wang, H.Z. The Influence of Dietary Energy Levels on the Production Performance, Rumen Fermentation and Intramuscular Fat Metabolism of Housed Fattening Yaks. Master's Thesis, Sichuan Agricultural University, Chengdu, China, 2015. (In Chinese)
39. Cosseau, C.; Romano-Bertrand, S.; Duplan, H.; Lucas, O.; Ingrassia, I.; Pigasse, C.; Roques, C.; Jumas-Bilak, E. Proteobacteria from the human skin microbiota: Species-level diversity and hypotheses. *One Health* **2016**, *2*, 33–41. [[CrossRef](#)] [[PubMed](#)]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.