



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Limosilactobacillus reuteri HM108 Alleviates Hyperuricemia in Honeybees through Degradation of Intestinal Purines.

Zonghan Zhou^{1,#}, Bin Liu^{2,3,#}, LuLiu^{2,3}, Junying Zhao^{2,3}, XianpingLi^{2,3},
Weicang Qiao^{2,3}, Li jun Chen^{1,2,3,*}

¹ Key Laboratory of Dairy Science, Ministry of Education, Food Science College, Northeast Agricultural University, Harbin, 150030, China;

² National Engineering Research Center of Dairy Health for Maternal and Child, Beijing Sanyuan Foods Co. Ltd., Beijing, 100163, China.

³ Beijing Engineering Research Center of Dairy, Beijing Technical Innovation Center of Human Milk Research, Beijing Sanyuan Foods Co. Ltd., Beijing 100163, China.

ABSTRACT: Hyperuricemia is a prevalent metabolic disorder associated with gout and systemic inflammation, underscoring the urgent need for safe and effective therapeutic strategies. Probiotics derived from human milk have garnered considerable interest due to their safety profile and potential urate-lowering effects. This study evaluated the ameliorative effects of human milk-derived *Limosilactobacillus reuteri* HM108 on hemolymph uric acid (UA) levels and intestinal inflammation using a hyperuricemic honeybee model, and further explored the underlying mechanisms. Newly emerged worker bees were divided into three groups: control (CON), hyperuricemia model (HUA, fed a yeast RNA diet), and HM108 intervention (HUA diet + HM108). After 12 days, hemolymph UA levels and intestinal inflammatory gene expression were assessed. Multi-omics approaches including metabolomics, transcriptomics, and metagenomics were integrated to investigate the mechanisms. The results showed that the HUA group had a significant increase in hemolymph UA (+33.19%, $P < 0.01$) and pronounced upregulation of pro-inflammatory genes (*Wnt-Rho1*, *PGRP-LC*, *Atg1*, *MAPK-phl*, *Toll*). HM108 intervention restored UA to CON levels and reversed the expression of inflammatory genes. Metabolomics revealed significant enrichment of purine/pyrimidine metabolism pathways and UA precursors (e.g., guanine, hypoxanthine) in the HUA group, which were significantly suppressed by HM108. Metagenomics confirmed successful colonization of HM108, whose abundance was negatively correlated with UA levels, and revealed that HM108 alleviated HUA-induced gut microbiota dysbiosis. In conclusion, HM108 ameliorates hyperuricemia by degrading intestinal purines, reducing UA synthesis, attenuating inflammation, and modulating gut microbiota, demonstrating its potential as a functional food or therapeutic agent.

Keywords: Hyperuricemia, *Limosilactobacillus reuteri* HM108, Purine metabolism, Gut microbiota

1. Introduction

Hyperuricemia is a metabolic disorder characterized by abnormally elevated serum uric acid levels[1]. It has become one of the most prevalent metabolic diseases worldwide, with a global prevalence ranging from 2.6% to 36%. Higher prevalence rates are generally observed in developed and middle-income countries. Reported data indicate that approximately 21% of adults in the United States are affected by

Contributed equally to this work.

*Corresponding author

chenlijun@sanyuan.com.cn

Received 9 October 2025

Received in revised form 20 November 2025

Accepted 27 February 2026

hyperuricemia[2], while the prevalence in the Japanese general population is approximately 25.8%[3]. Other developed countries and regions also show high prevalence rates, such as Australia (16.6%)[4], Finland (36.48%)[5], New Zealand (17.0%)[6], Ireland (24.5%)[7], Russia (16.8%)[8], Turkey (12.1%)[9], and Qatar (21.2%)[10]. Furthermore, the prevalence of hyperuricemia exhibits gender differences (higher in males than in females)[5–7,9] and regional variations (higher in coastal areas than inland areas). Studies indicate that by 2019, the pooled prevalence of hyperuricemia in mainland China was approximately 16.4%[11], with notable regional and gender disparities. Among Chinese individuals aged 20–29 years, the prevalence in males (21.5%) was twice that in females (8.9%)[11]. The highest prevalence was observed in southern China (25.5%) and southwestern China (21.2%), while the lowest was in northwestern China (12.6%)[11]. Additionally, between 2001 and 2017, the prevalence of hyperuricemia in China increased significantly from 8.5% to 18.4%, demonstrating a clear upward trend[11,12].

Hyperuricemia generally arises from an imbalance between uric acid (UA) production and excretion[13]. UA in the human body originates from two main routes: endogenous production and exogenous intake[14]. Endogenous accumulation is primarily determined by enzymes involved in purine metabolism, whereas exogenous intake mainly reflects excessive consumption of purine-rich foods[14,15]. Approximately 70% of UA is eliminated via the kidneys and about 30% via the intestine, and a growing body of evidence links alterations in the gut microbiota with hyperuricemia. Compared with healthy individuals, patients with hyperuricemia exhibit distinct gut microbial profiles[16]. Moreover, hyperuricemia-associated phenotypes can be transferred to germ-free recipients by fecal microbiota transplantation from hyperuricemic donors[17]. High UA levels are also tightly linked to systemic inflammation: UA can activate the NLRP3 inflammasome and promote release of proinflammatory cytokines such as interleukin-1 β , thereby driving systemic inflammation and related complications[18,19].

Conventional pharmacotherapy — for example, xanthine oxidase inhibitors (e.g., allopurinol, febuxostat) and uricosuric agents (e.g., benzbromarone, probenecid) — can be effective but is often accompanied by adverse effects such as rash, allergic reactions, and hepatic or renal toxicity in certain patient groups[20,21]. In recent years, probiotics have attracted substantial interest because some strains can reduce serum urate with relatively low side-effect profiles, offering promising therapeutic potential[22–25]. Several probiotic strains have already shown urate-lowering efficacy: for instance, *Limosilactobacillus fermentum* JL-3, isolated from the Chinese fermented vegetable product “jiangshui,” lowered serum UA in mice, alleviated UA-induced oxidative stress and inflammation, and modulated gut microbiota dysbiosis[26]. *Pediococcus acidilactici* GQ01 and related strains have been reported to decrease serum UA by inhibiting hepatic xanthine oxidase activity and upregulating renal ABCG2 transporter expression[27,28].

Breast milk-derived probiotics can promote immune maturation, enhance neonatal resistance to infection, modulate inflammatory responses, and reduce the risk of allergic and autoimmune conditions[29–31]. Certain strains isolated from human milk exhibit both anti-inflammatory and antioxidant activities, thereby protecting intestinal cells from oxidative damage[31]. Previous work has documented the

capacity of *Limosilactobacillus reuteri* MBHC 10138 to degrade purine nucleosides[32]. On this basis, we hypothesize that *Limosilactobacillus reuteri* HM108, isolated from Chinese breast milk, may mitigate hyperuricemia and associated inflammation by degrading purine substrates and thereby limiting UA synthesis.

The key to investigating the relationship between probiotic intervention and hyperuricemia lies in establishing an appropriate hyperuricemic animal model[33,34]. Similar to humans, honeybees possess purine and pyrimidine metabolic pathways and lack uricase activity[35–37]. In honeybees, ingested purines are broken down into hypoxanthine and xanthine, which are subsequently catalyzed by xanthine oxidase (XOD) to ultimately form uric acid—providing a theoretical basis for constructing a hyperuricemic model using honeybees. Furthermore, honeybees offer unique advantages as a hyperuricemic model: The honeybee provides a convenient model for dissecting host–microbe interactions because it harbors a relatively simple gut microbiota dominated by only five to nine core bacterial genera and germ-free individuals are readily obtainable. Using bees as an animal model allows clearer observation of microbe–host mechanisms. Therefore, the present study aimed to establish a hyperuricemic honeybee model to evaluate whether *L. reuteri* HM108 reduces serum urate levels and inflammation, and to elucidate the underlying mechanisms.

2. Materials and methods

2.1 Animals and Diet

Honeybees (*Apis mellifera*) used in this study were obtained from colonies maintained at the apiary of China Agricultural University. Selected pupae were placed in an incubator and kept overnight at 35 °C and 60–80% relative humidity. The following day, newly eclosed workers were checked; pupae that had not completed eclosion were returned to the incubator for an additional night. Fully eclosed bees were gently removed with forceps and allocated into disposable clear cups, 30 individuals per cup, with six cups in total. Worker gut homogenate was prepared from field-collected newly eclosed workers: the dissected guts were ground in phosphate-buffered saline (PBS) and mixed 1:1 with 50% glycerol to produce a worker gut fluid. To prepare the inoculum, 990 µL sterile PBS was mixed with 10 µL of the worker gut fluid, transferred into a sterile 2 mL microcentrifuge tube, and the remaining volume in the tube was filled with sterile 50% sucrose solution and vortexed to homogenize. Each cup of newly eclosed bees was fed one tube (aliquot) of this mixture and then returned to the incubator for 24 h (35 °C, 60–80% RH).

As demonstrated in multiple germ-free honeybee studies reported in the literature, while there are currently no explicit ethical guidelines specifically for insect experiments[38–40], the present study still adopted necessary measures to minimize potential distress of honeybees during the experimental process. During the experiment, honeybees were provided with adequate food and care on a daily basis. Prior to dissection, honeybees were euthanized via carbon dioxide (CO₂) anesthesia to maximize the reduction of their potential stress and discomfort.

Regular liquid diet: Five hundred grams of sucrose were dissolved in ultrapure water and the final volume adjusted to 1 L to obtain a 50% (w/v) sucrose solution. The solution was sterilized by filtration through a 0.22 µm membrane.

High-urate (model) liquid diet: To prepare the hyperuricemia-inducing liquid diet, 500 g sucrose and 5 g yeast-derived RNA powder were dissolved in ultrapure water and the volume adjusted to 1 L; the solution was then sterilized by 0.22 μm filtration to yield the experimental high-urate feeding solution.

Pollen solid diet: Canola pollen (sourced from Qinghai Province) was sterilized by electron-beam irradiation at a dose of 30 kGy for 4 h and subsequently ground to a fine powder.

Probiotic solid diet: The ground pollen was mixed with freeze-dried HM108 powder to obtain a pollen–bacteria mixture with a probiotic concentration of 1×10^{10} CFU/g. The bacterial powder was tested for viability prior to mixing to ensure strain activity and was stored at -20°C until being combined with pollen. The HM108 strain was isolated and identified by the Maternal and Infant Dairy Health Engineering Technology Research Center, Beijing Sanyuan Foods Co., Ltd., and deposited in the China General Microbiological Culture Collection Center (CGMCC) under accession number CGMCC No. 26166. The probiotic solid diet was prepared fresh each day to ensure bacterial viability.

2.2 Experimental design and sampling

A preliminary experiment was conducted prior to the main trial to establish a hyperuricemic honey bee model. Germ-free bees were obtained following a modified protocol based on the method described by Zheng et al.[41]. These germ-free bees were provided with worker bee gut homogenate within 24 hours post-eclosion to establish the initial gut microbiota. After 24 hours, the gut homogenate was removed, and the bees were assigned to dietary groups for the preliminary experiment. The preliminary control group was fed the regular liquid diet and pollen solid diet for 7 days, while the model group received the high-urate liquid diet along with pollen solid diet for the same period. The intervention duration was determined based on previous studies in honey bees[38–40,42]. Following the 7-day intervention, bees were sacrificed, hemolymph was collected, and uric acid concentration was measured. The results demonstrated that the hemolymph uric acid concentration in the model group was significantly higher than that in the control group (Supplementary Fig. 1), confirming the successful induction of hyperuricemia.

The formal experiment was subsequently conducted based on this validated model. After the initial 24 h post-eclosion period, the temporary inoculation mixture was removed and bees were assigned to experimental diets. The control group (CON) received the regular liquid diet together with the pollen solid diet for 12 consecutive days. The hyperuricemia group received the high-urate liquid diet plus the pollen solid diet for 12 days. The HM108 treatment group was fed the high-urate liquid diet and pollen solid diet for the first 7 days, followed by 5 days of the high-urate liquid diet combined with the probiotic solid diet. Throughout the experiment, bees were maintained at 35°C and 60–80% relative humidity and were supplied with sufficient food daily. Prior to sample collection, bees were euthanized by carbon dioxide to minimize unnecessary suffering.

2.3 Hemolymph uric acid quantification

Hemolymph was collected using a 10- μL pipettor (Eppendorf; Hamburg, Germany) from an incision above the median ocellus. Fresh hemolymph was snap-frozen in liquid nitrogen and stored at -80°C for

pending analysis. Hemolymph from 12 bees were pooled as one analytical sample. Each pooled sample was diluted 10-fold with deionized water and uric acid (UA) levels were quantified using a commercial UA assay kit (Boxbio, Beijing, China).

2.4 Collection of intestinal samples

Bees were dissected using forceps sterilized with 75% ethanol. The terminal abdominal segment was grasped and gently pulled until the segment detached from the body. The intact intestinal tract was then carefully extracted using forceps. Isolated intestinal samples were placed in centrifuge tubes, snap-frozen in liquid nitrogen, and stored at -80°C until further processing.

2.5 RNA extraction and quantitative PCR

The intestines of eight bees were designated as eight samples, with each sample subjected to three technical replicates. Each dissected intestinal tissue segment was homogenized using a plastic pestle. Total RNA was extracted from individual samples using RNA-easy Isolation Reagent (Vazyme, China) according to the manufacturer's protocol. cDNA synthesis was performed using HiScript III RT SuperMix for qPCR (Vazyme, China). Quantitative PCR (qPCR) was conducted in standard 96-well plates using ChamQ Universal SYBR qPCR Master Mix (Vazyme, China) on a QuantStudio™ 1 Real-Time PCR System (Thermo Fisher Scientific, Waltham, MA, USA). Primer sequences are provided in Supplementary Table 1. The *A. mellifera actin* gene was chosen as the control, and relative expression was analyzed using the $2^{-\Delta\Delta\text{CT}}$ method.

2.6 Non-targeted metabolomics analysis

Gut samples were analyzed by liquid chromatography–tandem mass spectrometry (LC-MS/MS) for non-targeted metabolomics. Sample preparation followed the method described by Elizabeth J. Want [43]; after processing, extracts were injected into the LC-MS/MS system for analysis.

Ultra-high-performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) analyses were performed at Novogene Co., Ltd. (Beijing, China) using a Vanquish UHPLC system (ThermoFisher, Germany) coupled to either an Orbitrap Q Exactive™ HF or an Orbitrap Q Exactive™ HF-X mass spectrometer (Thermo Fisher, Germany). Samples were separated on a Hypersil Gold column (100×2.1 mm, $1.9\ \mu\text{m}$) using a linear gradient program with a flow rate of $0.2\ \text{mL/min}$. Mobile phase A was 0.1% formic acid in water and mobile phase B was methanol for both positive and negative ion modes. The solvent program was set as follows: 2% B for 1.5 min; $2 \rightarrow 85\%$ B over 3 min; $85 \rightarrow 100\%$ B over 10 min; $100 \rightarrow 2\%$ B over 10.1 min; and 2% B until 12 min. The Q Exactive™ HF mass spectrometer operated in both positive and negative ion modes with a spray voltage of 3.5 kV, capillary temperature of 320°C , sheath gas flow of 35 psi, auxiliary gas flow of $10\ \text{L/min}$, S-lens RF level of 60, and auxiliary gas heater temperature of 350°C .

Raw data files generated by UHPLC-MS/MS were processed using Compound Discoverer 3.3 (CD3.3, ThermoFisher) to perform peak alignment, peak extraction and quantification for each metabolite. Peak intensities were normalized to total spectral intensity. The normalized data were used to predict molecular

formulas based on adduct ions, molecular ion peaks and fragment ions. Peak annotation and identification were achieved by matching against the mzCloud (<https://www.mzcloud.org/>), mzVault and MassList databases to obtain accurate qualitative and relative quantitative results.

Statistical analyses were conducted using R (R version 3.4.3) and Python (Python 2.7.6). When variables did not follow a normal distribution, relative peak areas were obtained by applying the following normalization: $\text{normalized_value} = \text{raw quantitative value} \div (\text{sum of metabolite quantities in sample} \div \text{sum of metabolite quantities in QC1})$. Compounds with a coefficient of variation (CV) > 30% across QC samples were excluded from further analysis. The resulting dataset provided metabolite identifications and relative quantifications.

Detected metabolites were annotated using the KEGG database (<https://www.genome.jp/kegg/pathway.html>), the Human Metabolome Database (HMDB; <https://hmdb.ca/metabolites>) and LIPIDMaps (<http://www.lipidmaps.org/>). MetaboAnalyst 6.0 was used for downstream metabolomic data analysis and visualization[44].

2.7 Transcriptomics

Total RNA was extracted from gut tissues using the Quick-RNA Miniprep kit (Zymo Research; Irvine, CA, USA). RNA integrity and potential degradation or contamination were monitored by electrophoresis on 1% agarose gels, and purity was assessed with a NanoPhotometer spectrophotometer (IMPLEN; CA, USA). RNA integrity was further evaluated using the RNA Nano 6000 Assay Kit on an Agilent 2100 Bioanalyzer system (Agilent Technologies; Santa Clara, CA, USA).

RNA sequencing libraries were prepared with the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs; Ipswich, MA, USA), and unique index sequences were added to distinguish individual samples. Indexed libraries were clustered on the GBC Cluster Generation System using the TruSeq PE Cluster Kit v6.0-cBot-HS (Illumina; San Diego, CA, USA), and sequencing was performed on an Illumina NovaSeq 6000 platform (Illumina; San Diego, CA, USA) to generate 150 bp paired-end reads.

Sequencing quality for individual samples was assessed with FastQC v0.11.5 using default parameters. The *Apis mellifera* reference genome (Amel_HAv3.1) index was built using HISAT2 v2.0.5, and quality-trimmed reads were aligned to the constructed index using HISAT2 v2.10 with default settings. Gene-level quantification was performed with HTSeq v0.72 in the “union” mode, which counts only reads that uniquely map to a single gene while discarding reads that map to multiple loci or overlap multiple genes.

To improve functional annotation of the *A. mellifera* reference, genome reannotation was performed. Of the 12,360 annotated honeybee genes, 6,589 were successfully assigned to KEGG Orthology (KO) entries, and the hierarchical pathway information from KEGG was extracted. Functional analysis of differentially expressed genes (DEGs) was conducted based on KO markers.

2.8 Metagenomics

Total gut DNA was extracted using the CTAB method[45]. A total of 24 honeybee gut samples were subjected to metagenomic sequencing. Sequencing libraries were prepared with the NEBNext Ultra™ II DNA

Library Prep Kit (New England Biolabs; Massachusetts, USA). Library quality was evaluated using a Qubit 3.0 fluorometer (Life Technologies; Grand Island, NY, USA) and an Agilent 4200 system (Agilent Technologies; Santa Clara, CA, USA). Libraries were sequenced on an Illumina HiSeq X-Ten platform to generate 150 bp paired-end reads.

Raw sequencing reads were adapter-trimmed and quality-controlled using fastp with parameters -q 20 -u 10[46]. Host-derived reads were removed by mapping reads to the *Apis mellifera* reference genome (GCA_003254395) using the BWA-MEM algorithm with parameters -t 4 -R -M[47]. Downstream metagenomic data analysis and visualization were performed using the MicrobiomeAnalyst 2.0 online platform[48].

2.9 Statistical analysis

Quantitative data were expressed as mean $\pm$ standard deviation (SD). Hemolymph uric acid concentrations and the relative expression levels of inflammatory markers were analyzed and visualized using GraphPad Prism 9.0 (GraphPad Software, La Jolla, CA, USA). Between-group differences were assessed by one-way analysis of variance (ANOVA), with Dunnett's test used for post hoc pairwise comparisons. Multiple testing was controlled using the false discovery rate (FDR) method. A two-sided P value < 0.05 was considered statistically significant.

Metabolomic and transcriptomic datasets were analyzed and visualized with the MetaboAnalyst 6.0 online platform[44]. Random forest modeling and weighted gene co-expression network analysis (WGCNA) were carried out using the ImageGP 2 online tool[49].

3. Results

3.1 HM108 reduced hemolymph urate and downregulated inflammation-related gene expression in honeybees

To evaluate the urate-lowering effect of HM108, hemolymph uric acid was measured on day 12 in all three treatment groups. Compared with the control (CON) group, the hyperuricemia (HUA) group exhibited a marked and statistically significant increase in hemolymph urate (Fig. 1A), confirming successful establishment of the high-urate bee model. Administration of *Limosilactobacillus reuteri* HM108 significantly decreased hemolymph urate relative to the HUA group (Fig. 1A); urate levels in the HM108 group were not significantly different from those in CON, indicating that HM108 effectively counteracted the hyperuricemic effect of the purine-rich diet.

We next examined intestinal expression of inflammation-related genes. Relative to CON, the HUA group showed significant or highly significant upregulation of *Wnt-Rho1*, *PGRP-LC*, *Atg1*, *MAPK-phl* and *Toll* (Fig. 1B–F). Compared with HUA, bees treated with HM108 displayed a significant or highly significant reduction in the relative expression of all five inflammatory markers, with levels returning to values comparable to CON (no significant differences versus CON) (Fig. 1B–F).

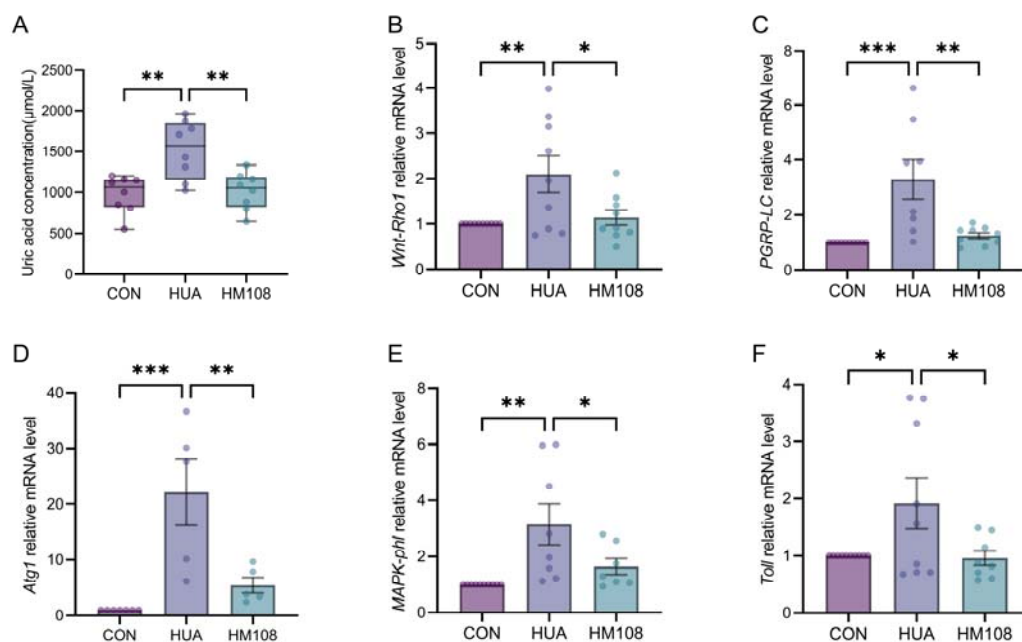


Figure 1. HM108 reduced hemolymph urate levels and modulated intestinal expression of inflammation-related genes. (A) Hemolymph uric acid concentration. (B–F) Relative mRNA expression levels of intestinal *Wnt-Rho1*, *PGRP-LC*, *Atg1*, *MAPK-phl*, and *Toll*. Data are presented as mean ± SD. Between-group differences were assessed by one-way ANOVA with Dunnett's post-hoc test. CON, control diet; HUA, high-urate (model) diet; HM108, *Limosilactobacillus reuteri* HM108 (high-urate diet + HM108 supplementation) diet. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

3.2 HM108 diet reduced intestinal precursors of uric acid synthesis

Non-targeted metabolomics of gut samples detected 1,029 metabolites in positive-ion mode and 699 metabolites in negative-ion mode. Permutational Multivariate Analysis of Variance (PERMANOVA) based on Euclidean distance revealed a highly significant separation among the three groups (Fig. 2A, $P < 0.01$). Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA) showed clear separation between HUA and CON, and between HM108 and HUA (Supplementary Fig. 2). OPLS-DA was used to screen differential metabolites with the criteria $VIP > 1$, $P < 0.05$ and fold change (FC) > 2 . Compared with CON, the HUA group had 270 upregulated and 194 downregulated differential metabolites (Fig. 2B). Compared with HUA, the HM108 group showed 37 upregulated and 66 downregulated metabolites (Fig. 2C). Cluster heatmaps of the intersecting differential metabolites for HUA vs. CON and HM108 vs. HUA (Fig. 2D, E) indicated that samples from the HM108 and CON groups clustered more closely, and that several metabolites elevated by the HUA diet were markedly reduced after HM108 supplementation. A random forest classifier also separated the three groups effectively (Supplementary Fig. 3; OOB error = 0.0833).

Relative to CON, the HUA group exhibited significant increases in multiple nucleic acid-related metabolites, including adenine nucleotides, adenosine, inosine monophosphate (IMP), inosine, hypoxanthine, xanthine nucleotides, guanine nucleotides, guanosine and guanine (Fig. 2F; Supplementary Fig. 4). Most of these nucleic-acid-related compounds were significantly decreased in the HM108 group versus HUA (Fig. 2F; Supplementary Fig. 4). In addition, hippuric acid—an important effector metabolite implicated in urate excretion—was significantly elevated in HUA compared with CON (Fig. 2G).

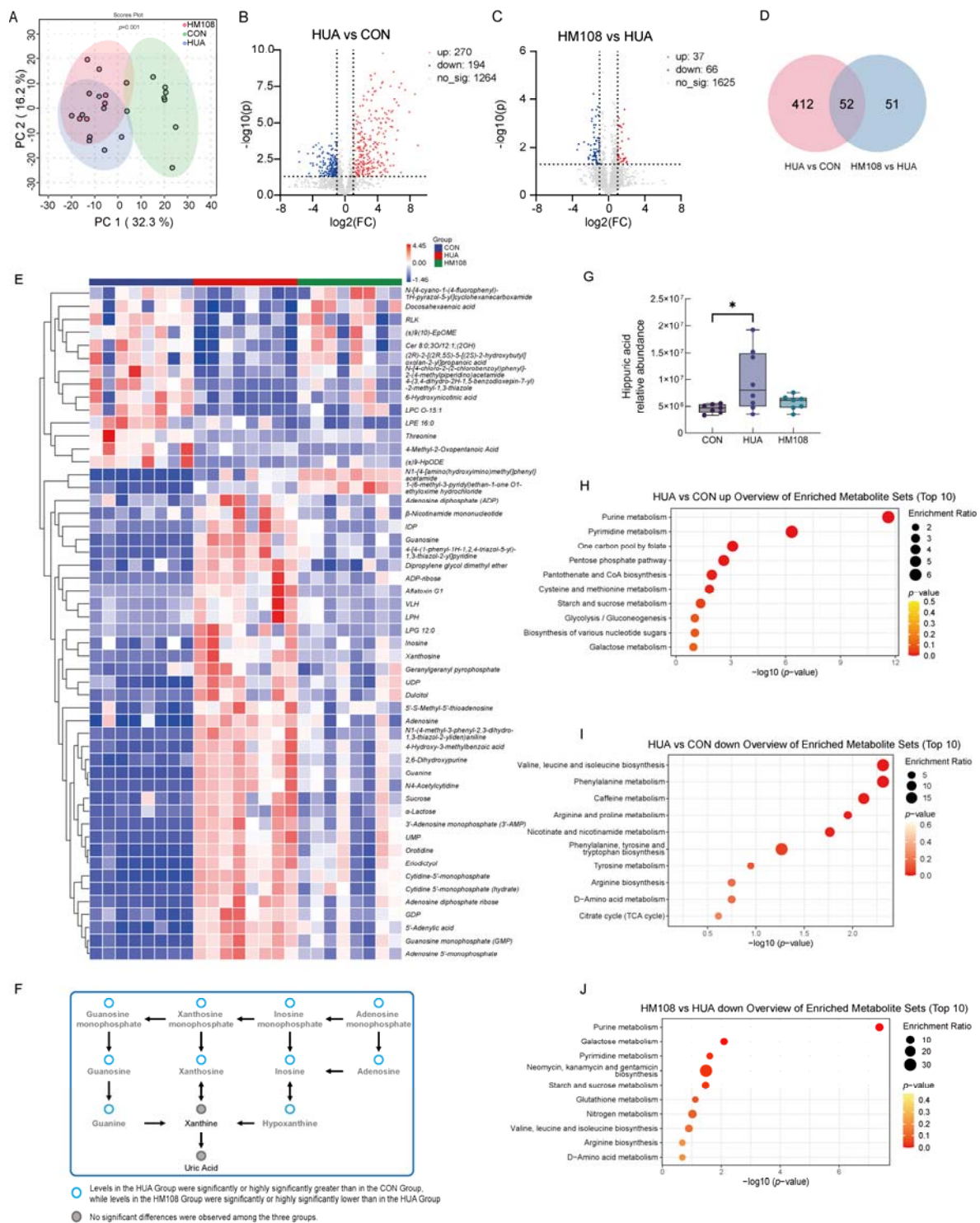


Figure 2. Effects of HM108 on the intestinal metabolome of honeybees. (A) PCA plot of untargeted metabolomics. (B) Volcano plot of HUA vs. CON. (C) Volcano plot of HM108 vs. HUA; (D) Venn diagram of differential metabolites; (E) Clustering heatmap of shared differential metabolites; (F) Abundance changes of purine catabolism-related metabolites; (G) Intestinal hippurate abundance; (H) Upregulated pathways in HUA vs. CON; (I) Downregulated pathways in HUA vs. CON; (J) Downregulated pathways in HM108 vs. HUA; CON, control diet; HUA, high-urate (model) diet; HM108, *Limosilactobacillus reuteri* HM108 (high-urate diet + HM108 supplementation) diet. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

To further explore pathway-level differences, we performed enrichment analysis in MetaboAnalyst 6.0 using the selected differential metabolites. Compared to the CON group, the HUA group exhibited significantly upregulated pathways including Purine metabolism, Pyrimidine metabolism, One carbon pool by folate, and the Pentose phosphate pathway (FDR $P < 0.05$; Fig. 2H). Significantly downregulated pathways in

the HUA group were Valine, leucine and isoleucine biosynthesis, Phenylalanine metabolism, Caffeine metabolism, Arginine and proline metabolism, and Nicotinate and nicotinamide metabolism (not significant after FDR correction; Fig. 2I). In comparison with the HUA group, the Purine metabolism pathway was significantly downregulated in the HM108 group (FDR $P < 0.05$; Fig. 2J).

3.3 HM108 diet modulated intestinal gene expression in honeybees

To probe the molecular basis underlying metabolic differences among the CON, HUA, and HM108 groups, we performed transcriptome profiling of gut tissue. Principal component analysis (PCA) did not reveal clear separation among the three groups (Fig. 3A). Differential expression analysis identified 379 differentially expressed genes (DEGs) in HUA versus CON, of which 83 were upregulated and 296 downregulated (Fig. 3B). In the HM108 versus HUA comparison, 145 DEGs were found, including 47 upregulated and 98 downregulated genes (Fig. 3C). Nineteen DEGs overlapped between the two comparisons (HUA vs CON and HM108 vs HUA) (Fig. 3D). Hierarchical clustering of these 19 genes showed marked divergence between HUA and CON that was largely attenuated in the HM108 group, suggesting that HM108 partially reversed HUA-induced transcriptomic perturbations (Fig. 3E).

KEGG enrichment of the DEGs (from volcano-plot selection) revealed that, relative to CON, HUA exhibited significant enrichment and upregulation of pathways including phenylalanine metabolism, tyrosine metabolism, tryptophan metabolism, and ECM–receptor interaction (Fig. 3F). Pathways significantly downregulated in HUA included glycosaminoglycan biosynthesis—chondroitin sulfate/dermatan sulfate, neuroactive ligand–receptor interaction, and the phosphatidylinositol signaling system (Fig. 3G). Compared with HUA, the HM108 group showed significant downregulation of thiamine metabolism, glycosaminoglycan biosynthesis—heparan sulfate/heparin, and folate biosynthesis.

A random forest classifier distinguished HUA from CON with an out-of-bag error rate of 0.312; the most informative features in this model included LOC100577231, LOC726309, and LOC550930 (Fig. 3I). Weighted gene co-expression network analysis (WGCNA) partitioned all transcripts into six modules; the yellow module demonstrated a negative correlation with hemolymph urate concentration that approached statistical significance ($p = 0.062$, Fig. 3J). The yellow module comprised 128 genes, of which 45 were downregulated in HUA relative to CON. KEGG enrichment of genes within the yellow module implicated pathways such as drug metabolism—cytochrome P450, metabolism of xenobiotics by cytochrome P450, nitrogen metabolism, and ribosome biogenesis in eukaryotes (Fig. 3K).

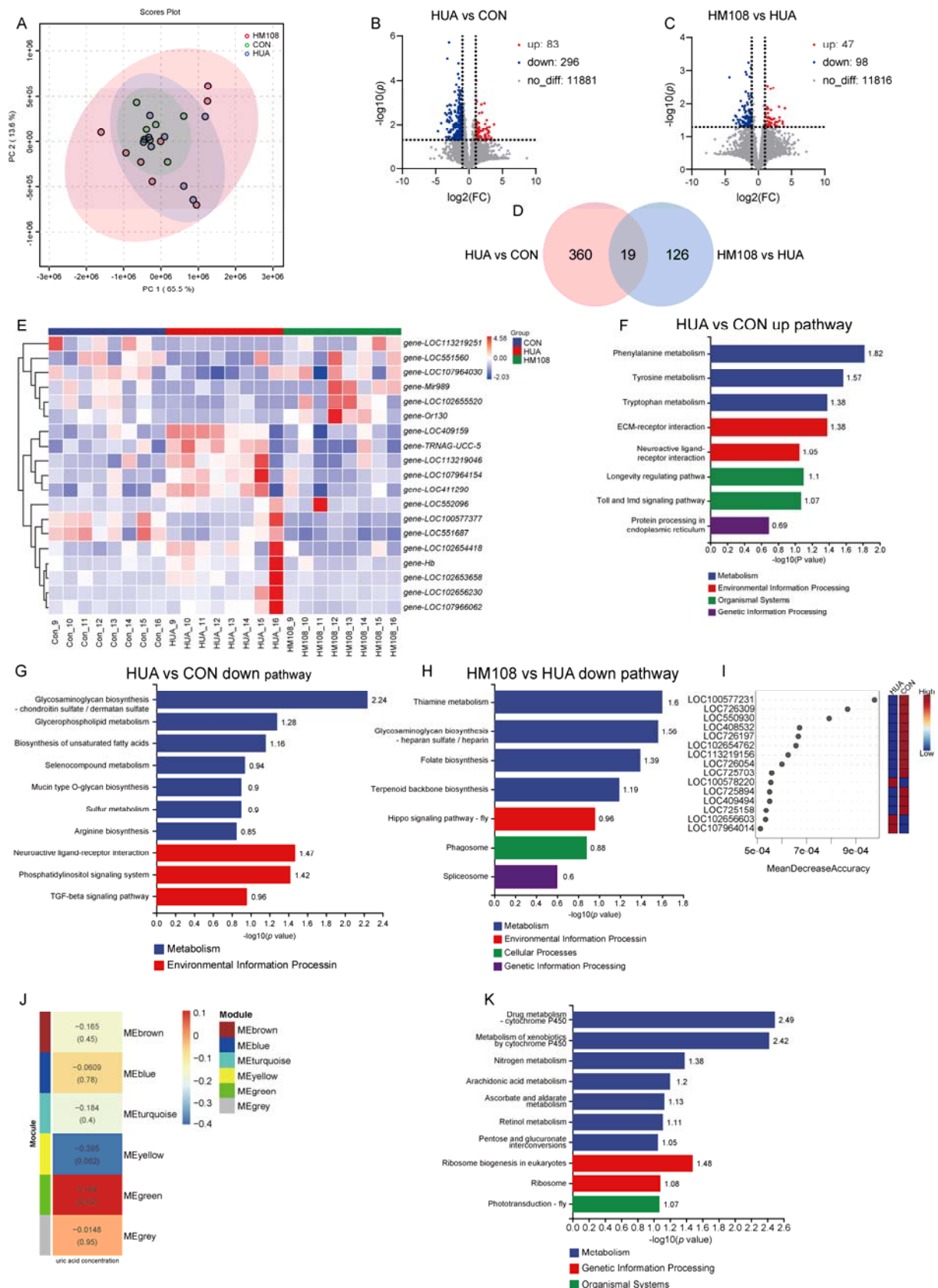


Figure 3. Effects of HM108 on the intestinal transcriptome of honeybees. (A) PCA plot of transcriptomics; (B) Volcano plot of HUA vs. CON; (C) Volcano plot of HM108 vs. HUA; (D) Venn diagram of differentially expressed genes (DEGs); (E) Clustering heatmap of shared DEGs; (F) Upregulated pathways in HUA vs. CON; (G) Downregulated pathways in HUA vs. CON; (H) Downregulated pathways in HM108 vs. HUA; (I) Top 15 informative features from random forest analysis of HUA vs. CON; (J) WGCNA analysis results; (K) Enriched pathways in the WGCNA yellow module. CON, control diet; HUA, high-urate (model) diet; HM108, *Limosilactobacillus reuteri* HM108 (high-urate diet + HM108 supplementation) diet.

3.4 HM108 Modulates the Honeybee Gut Microbiota

To further investigate the effects of a high-urate (HUA) diet and HM108 supplementation on gut microbes and host responses, we performed metagenomic sequencing of gut samples. Alpha-diversity metrics (Shannon and Simpson indices) were significantly higher in the HUA and HM108 groups than in the control (CON) group (Fig. 4A,B). Principal Coordinate Analysis (PCoA) combined with permutational multivariate analysis of variance (PERMANOVA) revealed significant differences among the three groups (PERMANOVA $p = 0.011$). The PCoA plot exhibited a distinct hierarchical clustering pattern with an inclusive relationship (HUA > HM108 > CON), and samples in the HM108 group clustered more closely with those in the CON group. These results suggest that HM108 can partially alleviate the alterations in gut microbiota structure induced by a hyperuricemic diet (Fig. 4C).

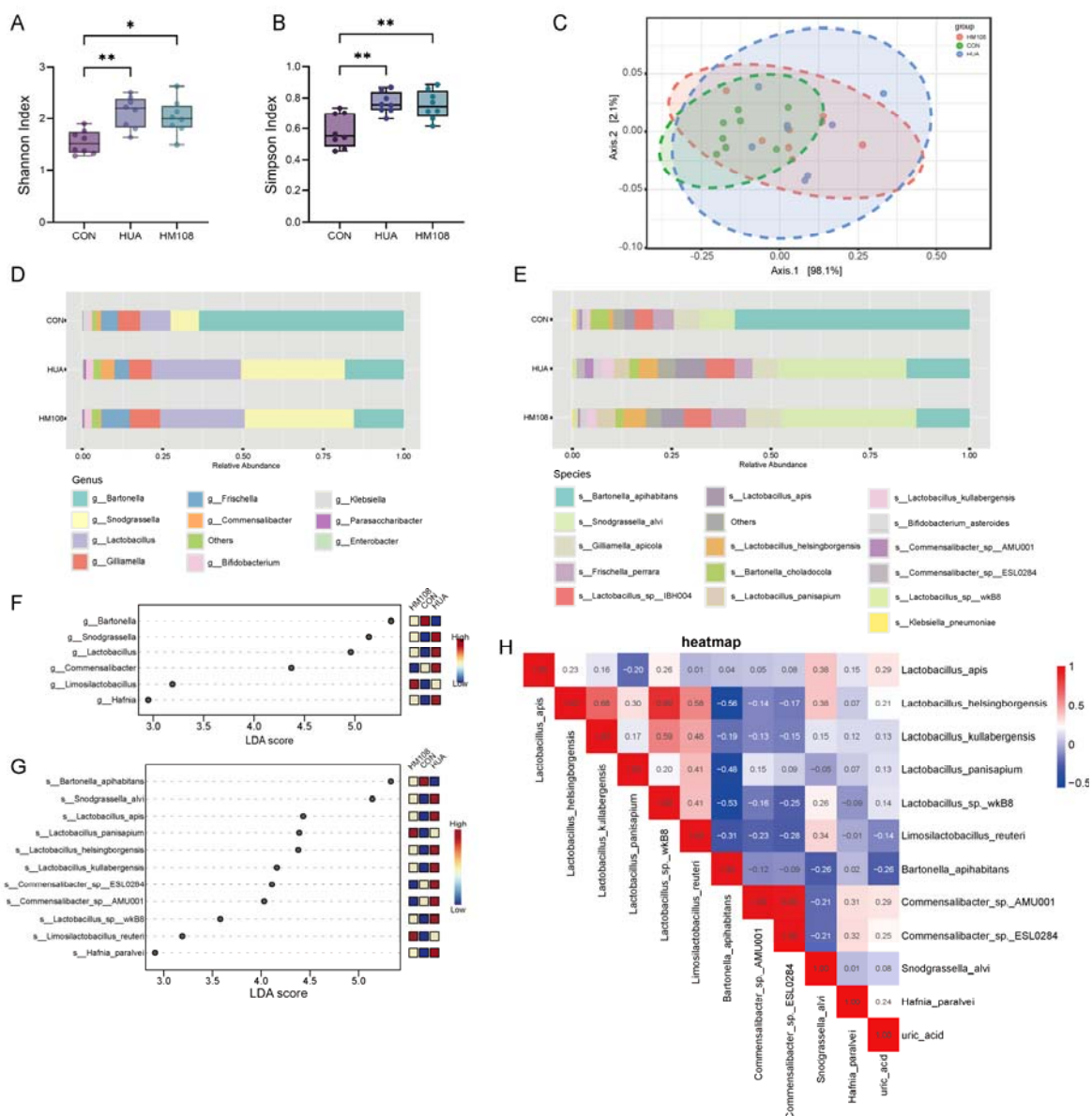


Figure 4. Effects of HM108 on the gut microbiota of honeybees. (A) Boxplot of Shannon index; (B) Boxplot of Simpson index; (C) PCoA plot; (D) Bar plot of microbial composition at the genus level; (E) Bar plot of microbial composition at the species level; (F) LEfSe analysis at the genus level; (G) LEfSe analysis at the species level; (H) Correlation analysis between differential taxa and urate concentration. CON, control diet; HUA, high-urate (model) diet; HM108, *Limosilactobacillus reuteri* HM108 (high-urate diet + HM108 supplementation) diet. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Taxonomic profiling at the genus and species levels showed *Bartonella*, *Lactobacillus*, and *Snodgrassella* as dominant genera across groups, although their relative abundances differed significantly among treatments (Fig. 4D). At species resolution, *Bartonella apihabitans* predominated in CON but its relative abundance declined in both HUA and HM108, whereas *Snodgrassella alvi* became dominant in the HUA and HM108 groups (Fig. 4E). LEfSe analysis identified six genera and eleven species with significantly different abundances (Fig. 4F,G). Notably, species including *Lactobacillus apis*, *Lactobacillus kullabergensis*, *Commensalibacter* sp. ESL0284, *Commensalibacter* sp. AMU001, and *Hafnia paralvei* were depleted in HUA but partially restored following HM108 treatment (Fig. 4G; Supplementary Fig. 5). *Bartonella apihabitans* was significantly reduced in both HUA and HM108 relative to CON (Supplementary Fig. 5), whereas *Limosilactobacillus reuteri* was markedly enriched in the HM108 group, indicating successful colonization (Supplementary Fig. 5). Spearman correlation analysis between LEfSe-identified taxa and hemolymph urate revealed negative associations for *B. apihabitans* and *L. reuteri* (Fig. 4H).

Functional profiling revealed pronounced differences between HUA and CON (Fig. 5A). Using limma with thresholds of $FC > 2$ and $P < 0.05$, we identified 307 upregulated and 556 downregulated functional genes in HUA versus CON (Fig. 5B), while HM108 versus HUA showed eight downregulated genes (Fig. 5C). A random forest classifier discriminated HUA from CON with an out-of-bag error of 0.25; key discriminatory KO features included K00411, K00564, and K07258 (Fig. 5D). KEGG enrichment indicated that, compared with CON, HUA exhibited significant upregulation of folate biosynthesis, purine metabolism, tyrosine metabolism, and nicotinate and nicotinamide metabolism (Fig. 5E). The KEGG enrichment analysis revealed that, compared to the CON group, the HUA group exhibited significant upregulation in the pathways of folate biosynthesis, purine metabolism, tyrosine metabolism, nicotinate and nicotinamide metabolism, glycine/serine/threonine metabolism, and biotin metabolism (Fig. 5E). Concurrently, pathways including porphyrin and chlorophyll metabolism, glyoxylate and dicarboxylate metabolism, glycine/serine/threonine metabolism, alanine/aspartate/glutamate metabolism, citrate cycle (TCA cycle), arginine biosynthesis, oxidative phosphorylation, phenylalanine/tyrosine/tryptophan biosynthesis, pyruvate metabolism, purine metabolism, fatty acid biosynthesis, and nitrogen metabolism were significantly downregulated (Fig. 5F). Compared with HUA, the HM108 group showed significant downregulation of arginine and proline metabolism (Supplementary Fig. 6). Collectively, these results indicate that HM108 supplementation partially restored microbial community structure and modulated functional potential related to purine metabolism and host–microbe interactions.

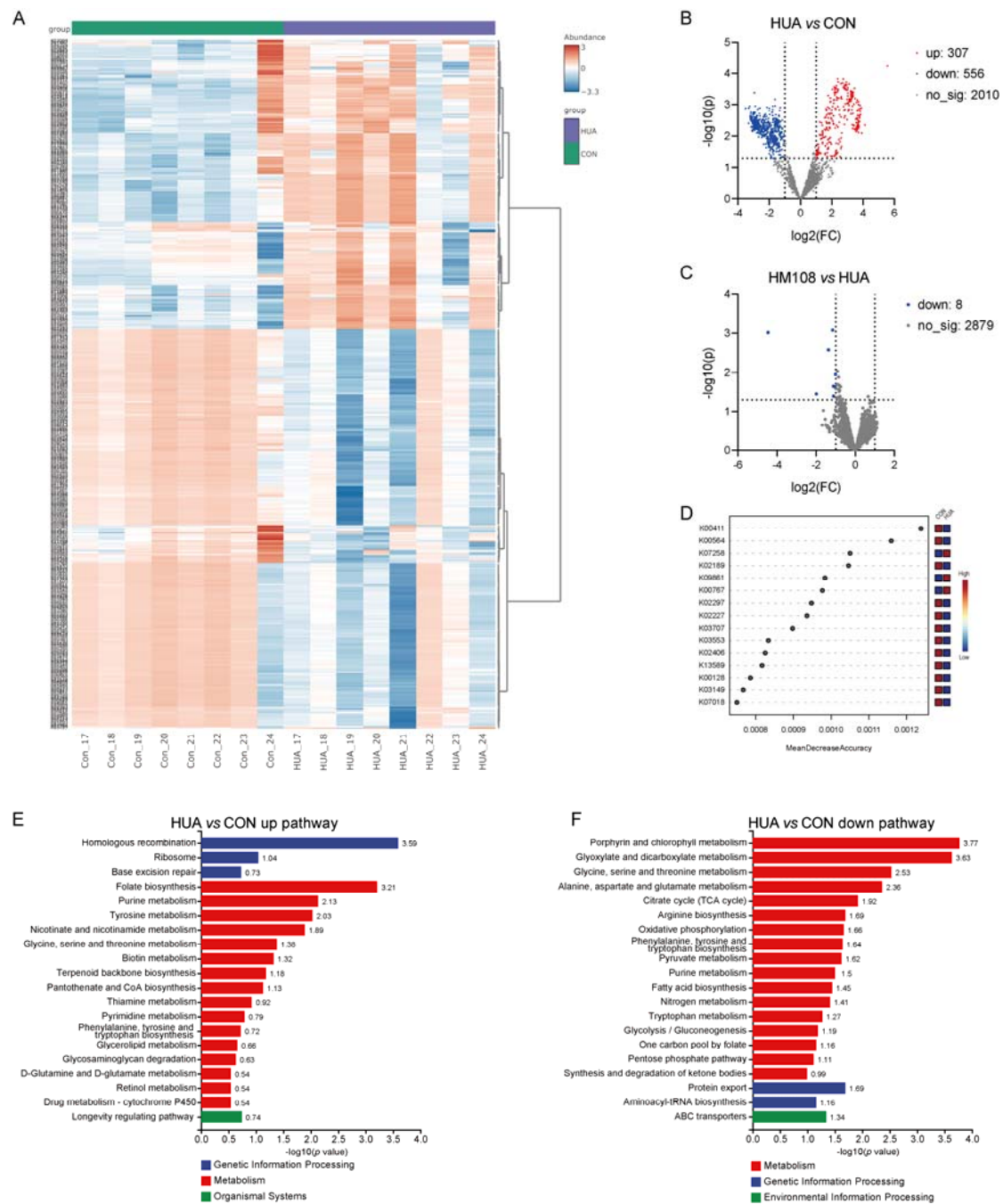


Figure 5. Effects of HM108 on gut microbiota function in honeybees. (A) Heatmap of gut microbial genes in HUA vs. CON; (B) Volcano plot of gut microbial genes in HUA vs. CON; (C) Volcano plot of gut microbial genes in HM108 vs. HUA; (D) Random forest analysis of top 15 informative features in HUA vs. CON; (E) Significantly upregulated pathways in HUA vs. CON; (F) Significantly downregulated pathways in HUA vs. CON. CON, control diet; HUA, high-urate (model) diet; HM108, *Limosilactobacillus reuteri* HM108 (high-urate diet + HM108 supplementation) diet. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4. Discussion

Hyperuricemia (HUA) is a global metabolic disorder associated with risk factors such as alcohol consumption, obesity, and hypertension[50–53]. Because conventional pharmacotherapies can produce adverse effects, probiotic approaches—being cost-effective and generally well tolerated—have gained increasing interest as potential adjuncts or alternatives for HUA management[23]. Prior studies demonstrated that certain probiotic strains mitigated HUA by reducing uric acid (UA) production or enhancing UA

excretion[32,54–57]. A high-purine diet is a significant contributing factor to hyperuricemia. This study specifically modeled this pathogenic factor using a bee model. In the present study, administration of *Limosilactobacillus reuteri* HM108—isolated from human breast milk—significantly reduced hemolymph UA and attenuated intestinal inflammation in a hyperuricemic honeybee model. Integrative analyses combining metagenomics, transcriptomics, and metabolomics were used to explore potential mechanisms underlying this effect.

UA is the final product of purine catabolism and a central etiological factor in HUA[58]. Hyperuricemia is frequently accompanied by impairment of the intestinal barrier and activation of inflammatory responses, manifested as decreased expression of epithelial tight-junction proteins (e.g., occludin, claudin-1, ZO-1), increased intestinal permeability, and elevated local and systemic proinflammatory cytokines (e.g., IL-1 β , TNF- α , IL-6)[59–61]. Previous work showed that administration of *Alistipes indistinctus* or *Limosilactobacillus fermentum* NCU003018 reduced serum UA by ~50% and ~30.8%, respectively, in murine HUA models[62,63]. Here, *L. reuteri* HM108 reduced hemolymph UA by 33.19%, indicating a pronounced urate-lowering effect. Recent evidence further indicates that urate can directly perturb immune cell populations—modifying cytokine expression, chemotaxis and differentiation—and thereby promote dysregulated inflammatory responses[18]. Consistent with this, the HUA diet upregulated intestinal expression of inflammatory genes including *Wnt-Rho1*, *PGRP-LC*, *Atg1*, *MAPK-phl*, and *Toll*, whereas HM108 supplementation restored these markers toward control levels, suggesting an anti-inflammatory action of the strain.

Metabolomics provided a comprehensive view of altered metabolic states. Both untargeted and targeted metabolomic approaches have been applied to HUA biomarker discovery[64], though clinical metabolomic profiles are often confounded by demographic and lifestyle variables (e.g., sex, age, geographic region, diet), which can complicate PCA interpretation[65]. Mirroring previous murine findings[66], we observed clear separation between HUA and CON metabolic profiles, and HM108 produced a distinct shift relative to HUA, with PCA and shared-metabolite clustering indicating that HM108 ameliorated HUA-induced metabolic perturbations. Compared with CON, HUA intestines exhibited upregulation of purine and pyrimidine metabolism (Fig. 2H) and marked accumulation of urate precursors (e.g., guanosinemonophosphate, guanine, guanosine, hypoxanthine; Fig. 2F), supporting the view that elevated hemolymph UA in HUA bees was largely driven by rapid increases in intestinal urate precursors. The reductions of these precursors in the HM108 group suggest that HM108 may decrease UA levels by degrading purine-related compounds or reducing intestinal purine absorption, consistent with reports that *L. reuteri* MBHC 10138 can metabolize purine nucleotides[32].

Among pathways enriched for HUA vs. CON differential metabolites, several—including purine metabolism, caffeine metabolism, valine/leucine/isoleucine biosynthesis, arginine and proline metabolism, and glycine/serine/threonine metabolism—have been repeatedly implicated in HUA-related metabolic alterations[66–68]. Notably, perturbations in arginine and proline metabolism have been observed across gut,

serum and urine matrices in HUA subjects[68]. Prior studies also reported that *Alistipes indistinctus*–derived hippuric acid enhanced intestinal UA excretion by promoting PPAR γ binding at the ABCG2 promoter, which aligns with the elevated hippuric acid detected in our HUA group. Given that honeybees rely exclusively on intestinal routes for urate elimination, increased intestinal UA in HUA bees likely imposed greater excretory pressure, potentially inducing adaptive upregulation of hippuric acid to facilitate UA clearance; the normalization of hippuric acid in HM108 bees was concordant with their lower hemolymph UA.

Hippuric acid is formed from benzoate and glycine, and intestinal phenylalanine provides an important precursor pool. Transcriptomic enrichment of phenylalanine metabolism in HUA paralleled elevations in hippuric acid. Aromatic amino acids (phenylalanine, tyrosine, tryptophan) generate metabolites—such as short-chain fatty acids and indole derivatives—that support barrier integrity and immune regulation[69,70], and hosts may upregulate AAA metabolism to compensate for dysbiotic loss of microbial functions. The extracellular matrix (ECM) sustains epithelial architecture and tight-junction integrity, while ECM–receptor interactions regulate epithelial differentiation, proliferation and repair[71–74]. Altered ECM–receptor signaling in HUA therefore suggested compromised barrier function and aligned with elevated inflammatory markers. Chondroitin sulfate has been implicated in preserving epithelial barrier integrity and mitigating oxidative and inflammatory damage[75]. Moreover, neuroactive ligand–receptor signaling (e.g., AHR, VIP) modulates mucosal immune cells, epithelial function and microbial homeostasis; downregulation of these pathways in HUA is consistent with reports linking HUA to barrier dysfunction[76–81]. Finally, the downregulation of thiamine metabolism observed in HM108 relative to HUA implied a reduction in intestinal energy demand following HM108 treatment, consistent with the elevated energy requirements evident in the HUA condition. [34]

Multiple studies have demonstrated a close association between the gut microbiota and hyperuricemia[82,83]. Previous findings on α -diversity in hyperuricemia have been inconsistent: some clinical trials reported no significant difference in the Shannon index[62], whereas animal studies frequently observed a marked reduction in model (HUA) groups relative to controls[66,84]. In the present study, the HUA and HM108 groups exhibited significantly higher Shannon and Simpson indices than the control (CON) group, indicating that the high-purine diet altered honeybee gut community structure. Further analysis of differentially abundant microorganisms among the three groups revealed that the abundance of multiple *Lactobacillus* species was significantly elevated in the HUA and HM108 groups, whereas the abundance of *Bartonella apihabitans* was markedly reduced (Fig. 4G, Supplementary Fig. 5). Correlation heatmap analysis demonstrated negative associations between *Bartonella apihabitans* and uric acid concentration, multiple *Lactobacillus* species, and *Snodgrassella* species (Fig. 4H). We therefore propose that the significant increase in alpha diversity observed in the HUA and HM108 groups may be attributed to the high-purine diet substantially suppressing the growth of the traditional dominant species, *Bartonella apihabitans*. This suppression likely provided a growth advantage to certain non-dominant genera (e.g., *Lactobacillus* and *Snodgrassella*), leading to their significant increase. Consequently, the previously

absolute dominance of *Bartonella* (exceeding 50% relative abundance) was supplanted by a more balanced, multi-genus community comprised of *Bartonella*, *Lactobacillus*, and *Snodgrassella*.

Principal coordinate analysis (PCoA) showed clear separation between HUA and CON, whereas the HM108 group clustered closer to HUA, suggesting that *Limosilactobacillus reuteri* HM108 partially mitigated HUA-induced community shifts.

Taxonomic profiling implicated *Bartonella* as an important contributor to intestinal tryptophan and phenylalanine synthesis and secretion in honeybees[85]. The marked decline of *Bartonella* in HUA paralleled transcriptomic enrichment of phenylalanine and tryptophan metabolism, implying that the host may compensate for dysbiosis by increasing aromatic amino acid (AAA) metabolism. Contrary to multiple reports of decreased *Lactobacillus* in HUA, we observed elevated *Lactobacillus* abundance in both HUA and HM108 groups, which may reflect the distinctive ecology of the honeybee gut. At species resolution, *Bartonella apihabitans* was depleted in HUA and HM108; prior work linked *B. apihabitans* to reduced abundance of certain honeybee gut viruses (e.g., AmFV)[86], suggesting that a high-purine diet could increase vulnerability to intestinal infection. Furthermore, the elevated abundance of *Lactobacillus reuteri* in the HM108 group may impose additional inhibitory effects on *Bartonella apihabitans* (the two show a negative correlation), offering a plausible explanation for its lowest observed abundance in this group. Notably, although the HM108 diet effectively restored hemolymph uric acid concentration to normal levels in bees, its capacity to reverse the gut microbiota alterations induced by the high-purine diet remained limited. The striking enrichment of *L. reuteri* in the HM108 group confirmed successful colonization by the administered strain, and its negative correlation with hemolymph uric acid supported a potential urate-lowering role. Although we did not observe the previously reported decline of *Alistipes indistinctus*, elevated intestinal levels of hippuric acid in HUA indicated upregulated urate-excretion-related metabolism and suggested the presence of enzyme systems capable of hippuric acid synthesis in honeybees—an avenue meriting further study.

Functional analyses revealed coherent shifts in microbial metabolic potential. Enrichment of purine metabolism in HUA suggested that the microbiota actively participated in degradation and reutilization of excess dietary purines; this observation was consistent with upregulation of folate biosynthesis (metagenome) and the one-carbon pool by folate pathway (metabolome). Excess purines can serve as rich carbon and nitrogen sources for gut microbes; their catabolism and recycling generate one-carbon units that depend on folate-mediated transfer and utilization. The conversion of purines to uric acid, catalyzed by xanthine oxidoreductase, simultaneously generates reactive oxygen species (ROS) such as superoxide anion and hydrogen peroxide[87]. Excessive ROS disrupt intestinal redox homeostasis, induce intestinal oxidative stress, and damage epithelial cells and tight junction proteins, thereby triggering or exacerbating gut inflammation[88]. In the HUA group, evidence of intestinal oxidative stress is supported by dysregulated amino acid metabolism in microbial functional genes, downregulation of the TCA cycle/oxidative phosphorylation, and elevated inflammatory factors (Figs. 1B-F, 5E, F)[89–91]. Under this condition, the upregulation of the pentose phosphate pathway (to enhance NADPH production for combating oxidative

stress) represents a widespread mechanism[92–96], and the same phenomenon has been observed in the gut microbiota of hyperuricemia patients[97]. Nicotinamide adenine dinucleotide (NAD⁺) and nicotinamide adenine dinucleotide phosphate (NADP⁺), generated through niacin and nicotinamide metabolism, play crucial roles in antioxidative processes. The upregulation of niacin and nicotinamide metabolic pathways in the metagenomic data of the HUA group suggests that the gut microbiota is actively responding to a state of oxidative stress. In the HUA group, the observed downregulation of the TCA cycle, oxidative phosphorylation, and pyruvate metabolism, coupled with the upregulation of tyrosine metabolism, indicates a shift in the energy production mode of the gut microbiota (Figs. 5E, F). This finding aligns with previous studies on high-purine-induced hyperuricemia or renal injury models, which consistently report that high-uric-acid groups rely more heavily on protein/amino acid and purine catabolism pathways for energy acquisition[98–100]. The results of the present study further corroborate this conclusion. Convergent enrichment of tyrosine metabolism in both metagenomic and transcriptomic data suggested activation of pathways associated with innate immune responses: tyrosine is the substrate for prophenoloxidase activation, which mediates pathogen encapsulation and killing, and tyrosine pathway upregulation is commonly observed in contexts of barrier impairment and immune activation[101–103].

Collectively, our multi-omics data suggested that excessive dietary purines remodeled gut community composition and functional capacity, disturbed one-carbon and energy metabolism, and thereby influenced host urate metabolism and intestinal homeostasis.

This study has several limitations. The differences in uric acid excretion mechanisms between humans and honeybees somewhat limit the extrapolation of this model for explaining the mechanism by which HM108 reduces hemolymph uric acid. Physiological differences between honeybees and humans preclude direct translation of the findings to humans, necessitating further validation in rodent models and clinical cohorts. Furthermore, the use of a single dose of HM108 in bees, the absence of an inactivated control group, and the lack of an additional experimental group with normal diet supplemented with HM108 intervention restrict conclusions regarding dose-dependency and whether the observed effects are attributable to the live probiotic. Future research should investigate the effects of different doses of *Limosilactobacillus reuteri* HM108, compare the functional differences between its viable and heat-inactivated forms, and administer the strain to healthy honeybees under standard feeding conditions. Such studies would clarify potential dose-dependent effects, the requirement for bacterial viability, and the regulatory impact of HM108 on the physiological status of healthy bees. Additionally, mRNA expression levels can be influenced by various post-transcriptional regulations, translational efficiency, and protein stability, potentially leading to discrepancies with functional protein levels. Therefore, relying solely on mRNA levels of inflammatory cytokines provides an incomplete assessment of inflammatory status. Subsequent studies should integrate proteomic analysis, ELISA, or Western Blot to quantitatively measure key inflammation-related proteins.

5. Conclusion

This study used a hyperuricemic honeybee model to evaluate the effects of *Limosilactobacillus reuteri* HM108, isolated from human breast milk, on hyperuricemia. The strain successfully colonized the honeybee gut, significantly reduced hemolymph uric acid levels, and attenuated intestinal inflammatory responses. Integrative metabolomic, transcriptomic, and metagenomic evidence suggested that HM108 may decrease host urate burden by degrading intestinal purine nucleotides and modulating microbial functional potential, thereby reducing the availability of purine substrates to the host and promoting restoration of gut homeostasis. Collectively, HM108 shows promise as a candidate for development as a functional food ingredient or adjunctive therapeutic agent, but its translation will require comprehensive follow-up validation.

Declaration of competing interest

The authors declare no conflict of interest./Authors Bin Liu, LuLiu, Junying Zhao, Xianping Li and Weicang Qiao were employed by Beijing Sanyuan Foods Co., Ltd. Other authors declared no conflicts of interest.

Acknowledgements

This work was supported by Beijing Science and Technology Plan (Grant No. Z221100006422012) , Beijing Innovation Team of Livestock Industry Technology System (Grant No. BAIC05), Beijing Capital Agribusiness & Foods Group Science and Technology Project (SNSPKJ (2022) 03).

References

- [1] L. Du, Y. Zong, H. Li, Q. Wang, L. Xie, B. Yang, Y. Pang, C. Zhang, Z. Zhong, J. Gao, Hyperuricemia and its related diseases: Mechanisms and advances in therapy, *Sig Transduct Target Ther* 9 (2024) 212. <https://doi.org/10.1038/s41392-024-01916-y>.
- [2] Y. Zhu, B.J. Pandya, H.K. Choi, Prevalence of gout and hyperuricemia in the US general population: The national health and nutrition examination survey 2007-2008: prevalence of gout and hyperuricemia in the US, *Arthritis & Rheumatism* 63 (2011) 3136–3141. <https://doi.org/10.1002/art.30520>.
- [3] J. Huang, Z.F. Ma, Y. Zhang, Z. Wan, Y. Li, H. Zhou, A. Chu, Y.Y. Lee, Geographical distribution of hyperuricemia in mainland China: A comprehensive systematic review and meta-analysis, *Glob Health Res Policy* 5 (2020) 52. <https://doi.org/10.1186/s41256-020-00178-9>.
- [4] K. Pathmanathan, P.C. Robinson, C.L. Hill, H.I. Keen, The prevalence of gout and hyperuricaemia in australia: An updated systematic review, *Seminars in Arthritis and Rheumatism* 51 (2021) 121–128. <https://doi.org/10.1016/j.semarthrit.2020.12.001>.
- [5] J. Timsans, J.E. Kauppi, A.M. Kerola, T.M. Lehto, H. Kautiainen, M.J. Kauppi, Hyperuricaemia: Prevalence and association with mortality in an elderly finnish population, *BMJ Open* 13 (2023) e072110. <https://doi.org/10.1136/bmjopen-2023-072110>.
- [6] L.K. Stamp, J.E. Wells, S. Pitama, A. Faatoese, R.N. Doughty, G. Whalley, A.M. Richards, V.A. Cameron, Hyperuricaemia and gout in font-variant:Small-caps; ew Zealand rural and urban M āori and non- M āori communities, *Internal Medicine Journal* 43 (2013) 678–684. <https://doi.org/10.1111/imj.12062>.
- [7] A. Kumar A. U., L.D. Browne, X. Li, F. Adeeb, F. Perez-Ruiz, A.D. Fraser, A.G. Stack, Temporal trends in hyperuricaemia in the irish health system from 2006-2014: A cohort study, *PLoS ONE* 13 (2018) e0198197. <https://doi.org/10.1371/journal.pone.0198197>.
- [8] S.A. Maksimov, S.A. Shalnova, Y.A. Balanova, A.D. Deyev, S.E. Evstifeyeva, A.E. Imayeva, A.V. Kapustina, N.S. Karamnova, G.A. Muromtseva, O.P. Rotar, E.V. Shlyakhoto, S.A. Boytsov, O.M. Drapkina, Hyperuricemia versus lifestyle in men and women of the russian federation population, *Russ Open Med J* 9 (2020) e0214. <https://doi.org/10.15275/rusomj.2020.0214>.

- [9] I. Sari, S. Akar, B. Pakoz, A.R. Sisman, O. Gurler, M. Birlik, F. Onen, N. Akkoc, Hyperuricemia and its related factors in an urban population, izmir, turkey, *Rheumatol Int* 29 (2009) 869–874. <https://doi.org/10.1007/s00296-008-0806-2>.
- [10] Y. Al Shanableh, Y.Y. Hussein, A.H. Saidwali, M. Al-Mohannadi, B. Aljalham, H. Nurulhoque, F. Robelah, A. Al-Mansoori, S.M. Zughaier, Prevalence of asymptomatic hyperuricemia and its association with prediabetes, dyslipidemia and subclinical inflammation markers among young healthy adults in qatar, *BMC Endocr Disord* 22 (2022) 21. <https://doi.org/10.1186/s12902-022-00937-4>.
- [11] Y. Li, Z. Shen, B. Zhu, H. Zhang, X. Zhang, X. Ding, Demographic, regional and temporal trends of hyperuricemia epidemics in mainland China from 2000 to 2019: A systematic review and meta-analysis, *Global Health Action* 14 (2021) 1874652. <https://doi.org/10.1080/16549716.2021.1874652>.
- [12] M. Zhang, X. Zhu, J. Wu, Z. Huang, Z. Zhao, X. Zhang, Y. Xue, W. Wan, C. Li, W. Zhang, L. Wang, M. Zhou, H. Zou, L. Wang, Prevalence of hyperuricemia among Chinese adults: Findings from two nationally representative cross-sectional surveys in 2015–16 and 2018–19, *Front. Immunol.* 12 (2022) 791983. <https://doi.org/10.3389/fimmu.2021.791983>.
- [13] N. Liu, H. Xu, Q. Sun, X. Yu, W. Chen, H. Wei, J. Jiang, Y. Xu, W. Lu, The role of oxidative stress in hyperuricemia and xanthine oxidoreductase (XOR) inhibitors, *Oxidative Medicine and Cellular Longevity* 2021 (2021) 1470380. <https://doi.org/10.1155/2021/1470380>.
- [14] L.B. Sorensen, D.J. Levinson, Origin and extrarenal elimination of uric acid in man, *Nephron* 14 (1975) 7–20. <https://doi.org/10.1159/000180432>.
- [15] H.K. Choi, K. Atkinson, E.W. Karlson, W. Willett, G. Curhan, Purine-rich foods, dairy and protein intake, and the risk of gout in men, *N Engl J Med* 350 (2004) 1093–1103. <https://doi.org/10.1056/NEJMoa035700>.
- [16] J. Wei, Y. Zhang, N. Dalbeth, R. Terkeltaub, T. Yang, Y. Wang, Z. Yang, J. Li, Z. Wu, C. Zeng, G. Lei, Association between gut microbiota and elevated serum urate in two independent cohorts, *Arthritis & Rheumatology* 74 (2022) 682–691. <https://doi.org/10.1002/art.42009>.
- [17] J. Wang, Y. Chen, H. Zhong, F. Chen, J. Regenstein, X. Hu, L. Cai, F. Feng, The gut microbiota as a target to control hyperuricemia pathogenesis: Potential mechanisms and therapeutic strategies, *Critical Reviews in Food Science and Nutrition* 62 (2022) 3979–3989. <https://doi.org/10.1080/10408398.2021.1874287>.
- [18] D. Li, S. Yuan, Y. Deng, X. Wang, S. Wu, X. Chen, Y. Li, J. Ouyang, D. Lin, H. Quan, X. Fu, C. Li, W. Mao, The dysregulation of immune cells induced by uric acid: Mechanisms of inflammation associated with hyperuricemia and its complications, *Front. Immunol.* 14 (2023) 1282890. <https://doi.org/10.3389/fimmu.2023.1282890>.
- [19] W. Gu, J. Zhao, Y. Xu, Hyperuricemia-induced complications: Dysfunctional macrophages serve as a potential bridge, *Front. Immunol.* 16 (2025) 1512093. <https://doi.org/10.3389/fimmu.2025.1512093>.
- [20] R.R. Hemnani, S.V. Sood, S.D. Gupta, S.D. Malhotra, Allopurinol-induced hypersensitivity reaction in a patient of underlying renal impairment, *National Journal of Pharmacology and Therapeutics* 2 (2024) 41–44. https://doi.org/10.4103/NJPT.NJPT_50_23.
- [21] Y.-Y. Tien, M.-C. Shih, C.-P. Tien, H.-K. Huang, Y.-K. Tu, To Treat or Not to Treat? Effect of Urate-Lowering Therapy on Renal Function, Blood Pressure and Safety in Patients with Asymptomatic Hyperuricemia: A Systematic Review and Network Meta-Analysis, *J Am Board Fam Med* 35 (2022) 140–151. <https://doi.org/10.3122/jabfm.2022.01.210273>.
- [22] Y. Yang, J. Zhang, Q. Zhou, Targets and mechanisms of dietary anthocyanins to combat hyperglycemia and hyperuricemia: A comprehensive review, *Critical Reviews in Food Science and Nutrition* 62 (2022) 1119–1143. <https://doi.org/10.1080/10408398.2020.1835819>.
- [23] H. Zhao, Z. Lu, Y. Lu, The potential of probiotics in the amelioration of hyperuricemia, *Food Funct.* 13 (2022) 2394–2414. <https://doi.org/10.1039/D1FO03206B>.
- [24] Z.-P. Zou, J.-L. Li, Y.-F. Zhang, Y. Zhou, B.-C. Ye, Empowering probiotics with high xanthine transport for effective hyperuricemia management, *Gut Microbes* 16 (2024) 2399213. <https://doi.org/10.1080/19490976.2024.2399213>.
- [25] Q. Wang, J. Liang, Q. Zou, W. Wang, G. Yan, R. Guo, T. Yuan, Y. Wang, X. Liu, Z. Liu, Tryptophan metabolism-regulating probiotics alleviate hyperuricemia by protecting the gut barrier integrity and enhancing colonic uric acid excretion, *J. Agric. Food Chem.* 72 (2024) 26746–26761. <https://doi.org/10.1021/acs.jafc.4c07716>.

- [26] Y. Wu, Z. Ye, P. Feng, R. Li, X. Chen, X. Tian, R. Han, A. Kakade, P. Liu, X. Li, *Limosilactobacillus fermentum* JL-3 isolated from “jiangshui” ameliorates hyperuricemia by degrading uric acid, *Gut Microbes* 13 (2021) 1897211. <https://doi.org/10.1080/19490976.2021.1897211>.
- [27] X. Zhang, J. Jiang, J. Xin, N. Sun, Z. Zhao, B. Gan, Y. Jiang, X. Gong, H. Li, Hailin, X. Ni, Y. Chen, Y. Bai, H. Wang, Preventive effect of *Lactobacillus johnsonii* YH1136 against uric acid accumulation and renal damages., *Frontiers in Microbiology* 15 (2024) 1364857. <https://doi.org/10.3389/fmicb.2024.1364857>.
- [28] L. Ren, S. Wang, S. Liu, H.A.C. Prasanthi, Y. Li, J. Cao, F. Zhong, L. Guo, F. Lu, X. Luo, Postbiotic of *Pediococcus acidilactici* GQ01, a novel probiotic strain isolated from natural fermented wolfberry, attenuates hyperuricaemia in mice through modulating uric acid metabolism and gut microbiota, *Foods* 13 (2024) 923. <https://doi.org/10.3390/foods13060923>.
- [29] B.K. Alemu, G.G. Azeze, L. Wu, S.L. Lau, C.C. Wang, Y. Wang, Effects of maternal probiotic supplementation on breast milk microbiome and infant gut microbiome and health: A systematic review and meta-analysis of randomized controlled trials, *American Journal of Obstetrics & Gynecology MFM* 5 (2023) 101148. <https://doi.org/10.1016/j.ajogmf.2023.101148>.
- [30] K.E. Lyons, C.A. Ryan, E.M. Dempsey, R.P. Ross, C. Stanton, Breast milk, a source of beneficial microbes and associated benefits for infant health, *Nutrients* 12 (2020) 1039. <https://doi.org/10.3390/nu12041039>.
- [31] Y. Wang, B. Rui, X. Ze, Y. Liu, D. Yu, Y. Liu, Z. Li, Y. Xi, X. Ning, Z. Lei, J. Yuan, L. Li, X. Zhang, W. Li, Y. Deng, J. Yan, M. Li, Sialic acid-based probiotic intervention in lactating mothers improves the neonatal gut microbiota and immune responses by regulating sialylated milk oligosaccharide synthesis via the gut–breast axis, *Gut Microbes* 16 (2024) 2334967. <https://doi.org/10.1080/19490976.2024.2334967>.
- [32] J. Cheng, J.-H. Cho, J.-W. Suh, Characterization of human breast milk-derived *limosilactobacillus reuteri* MBHC 10138 with respect to purine degradation, anti-biofilm, and anti-lipid accumulation activities, *Antibiotics* 13 (2024) 964. <https://doi.org/10.3390/antibiotics13100964>.
- [33] Y. Wang, H. Zhang, S. Liu, S. Sun, W. Ren, T. Wang, S. Zhang, H. Yao, C. Jin, N. Wu, Comparative analysis of hyperuricemia induction methods and probiotic interventions in mice, *Current Research in Microbial Sciences* 9 (2025) 100422. <https://doi.org/10.1016/j.crmicr.2025.100422>.
- [34] S. Zhang, K. Li, H. Zhang, T. Fu, Y. Ma, S. Zhang, G. Xing, Y. Tong, Progress in animal models for studying hyperuricemia, *Front. Pharmacol.* 16 (2025) 1636205. <https://doi.org/10.3389/fphar.2025.1636205>.
- [35] X. Shi, J. Shi, L. Yu, X. Wu, Metabolic profiling of *Apis mellifera* larvae treated with sublethal acetamiprid doses, *Ecotoxicology and Environmental Safety* 254 (2023) 114716. <https://doi.org/10.1016/j.ecoenv.2023.114716>.
- [36] J. Gao, Y. Yang, S. Ma, F. Liu, Q. Wang, X. Wang, Y. Wu, L. Zhang, Y. Liu, Q. Diao, P. Dai, Combined transcriptome and metabolite profiling analyses provide insights into the chronic toxicity of carbaryl and acetamiprid to *Apis mellifera* larvae, *Sci Rep* 12 (2022) 16898. <https://doi.org/10.1038/s41598-022-21403-0>.
- [37] R. Pavlović, S. Stojanović, M. Pavlović, N. Drulović, M. Vujčić, B. Dojnov, Z. Vujčić, Glycosidase isoforms in honey and the honey bee (*Apis mellifera* L.): Differentiating bee- and yeast-derived enzymes and implications for honey authentication, *Insects* 16 (2025) 622. <https://doi.org/10.3390/insects16060622>.
- [38] Z. Zhang, X. Mu, Q. Cao, Y. Shi, X. Hu, H. Zheng, Honeybee gut *Lactobacillus* modulates host learning and memory behaviors via regulating tryptophan metabolism, *Nat Commun* 13 (2022) 2037. <https://doi.org/10.1038/s41467-022-29760-0>.
- [39] J. Wang, H. Lang, W. Zhang, Y. Zhai, L. Zheng, H. Chen, Y. Liu, H. Zheng, Stably transmitted defined microbial community in honeybees preserves *Hafnia alvei* inhibition by regulating the immune system, *Front Microbiol* 13 (2022) 1074153. <https://doi.org/10.3389/fmicb.2022.1074153>.
- [40] Z. Zhang, X. Mu, Y. Shi, H. Zheng, Distinct roles of honeybee gut bacteria on host metabolism and neurological processes, *Microbiol Spectr* 10 (2022) e02438-21. <https://doi.org/10.1128/spectrum.02438-21>.
- [41] H. Zheng, J. Perreau, J.E. Powell, B. Han, Z. Zhang, W.K. Kwong, S.G. Tringe, N.A. Moran, Division of labor in honey bee gut microbiota for plant polysaccharide digestion, *Proc Natl Acad Sci U S A* 116 (2019) 25909–25916. <https://doi.org/10.1073/pnas.1916224116>.
- [42] H. Lang, H. Duan, J. Wang, W. Zhang, J. Guo, X. Zhang, X. Hu, H. Zheng, Specific strains of honeybee gut *Lactobacillus* stimulate host immune system to protect against pathogenic *Hafnia alvei*, *Microbiol Spectr* 10 (2022) e01896-21. <https://doi.org/10.1128/spectrum.01896-21>.

- [43] E.J. Want, P. Masson, F. Michopoulos, I.D. Wilson, G. Theodoridis, R.S. Plumb, J. Shockcor, N. Loftus, E. Holmes, J.K. Nicholson, Global metabolic profiling of animal and human tissues via UPLC-MS, *Nat Protoc* 8 (2013) 17–32. <https://doi.org/10.1038/nprot.2012.135>.
- [44] Z. Pang, Y. Lu, G. Zhou, F. Hui, L. Xu, C. Viau, A.F. Spigelman, P.E. MacDonald, D.S. Wishart, S. Li, J. Xia, MetaboAnalyst 6.0: Towards a unified platform for metabolomics data processing, analysis and interpretation, *Nucleic Acids Research* 52 (2024) W398–W406. <https://doi.org/10.1093/nar/gkae253>.
- [45] W.K. Kwong, L.A. Medina, H. Koch, K.-W. Sing, E.J.Y. Soh, J.S. Ascher, R. Jaffé, N.A. Moran, Dynamic microbiome evolution in social bees, *Sci. Adv.* 3 (2017) e1600513. <https://doi.org/10.1126/sciadv.1600513>.
- [46] S. Chen, Y. Zhou, Y. Chen, J. Gu, fastp: An ultra-fast all-in-one FASTQ preprocessor, *Bioinformatics* 34 (2018) i884–i890. <https://doi.org/10.1093/bioinformatics/bty560>.
- [47] H. Li, R. Durbin, Fast and accurate long-read alignment with burrows–wheeler transform, *Bioinformatics* 26 (2010) 589–595. <https://doi.org/10.1093/bioinformatics/btp698>.
- [48] Y. Lu, G. Zhou, J. Ewald, Z. Pang, T. Shiri, J. Xia, MicrobiomeAnalyst 2.0: Comprehensive statistical, functional and integrative analysis of microbiome data, *Nucleic Acids Research* 51 (2023) W310–W318. <https://doi.org/10.1093/nar/gkad407>.
- [49] T. Chen, Y. Liu, T. Chen, M. Yang, S. Fan, M. Shi, B. Wei, H. Lv, W. Cao, C. Wang, J. Cui, J. Zhao, Y. Han, J. Xi, Z. Zheng, L. Huang, ImageGP 2 for enhanced data visualization and reproducible analysis in biomedical research, *iMeta* 3 (2024) e239. <https://doi.org/10.1002/imt2.239>.
- [50] M. Chen-Xu, C. Yokose, S.K. Rai, M.H. Pillinger, H.K. Choi, Contemporary prevalence of gout and hyperuricemia in the united states and decadal trends: The national health and nutrition examination survey, 2007–2016, *Arthritis & Rheumatology* 71 (2019) 991–999. <https://doi.org/10.1002/art.40807>.
- [51] G.F. Falasca, Metabolic diseases: Gout, *Clinics in Dermatology* 24 (2006) 498–508. <https://doi.org/10.1016/j.clindermatol.2006.07.015>.
- [52] S. Ebrahimpour-koujan, P. Saneei, B. Larijani, A. Esmailzadeh, Consumption of sugar sweetened beverages and dietary fructose in relation to risk of gout and hyperuricemia: A systematic review and meta-analysis, *Critical Reviews in Food Science and Nutrition* 60 (2020) 1–10. <https://doi.org/10.1080/10408398.2018.1503155>.
- [53] K. Qiao, X. Zhou, L. Zhang, W. Zhang, B. Sun, Y. Zhang, Metabolic mechanism of dietary factors and effect of dietary types associated with hyperuricemia: A review, *Food Science and Human Wellness* 14 (2025) 9250054. <https://doi.org/10.26599/fshw.2024.9250054>.
- [54] R. Shi, J. Ye, H. Fan, C. Xiao, D. Wang, B. Xia, Z. Zhao, B. Zhao, X. Dai, X. Liu, *Lactobacillus plantarum* LLY-606 supplementation ameliorates hyperuricemia via modulating intestinal homeostasis and relieving inflammation, *Food Funct.* 14 (2023) 5663–5677. <https://doi.org/10.1039/d2fo03411e>.
- [55] Z. Wang, Y. Huang, T. Yang, L. Song, Y. Xiao, Y. Chen, M. Chen, M. Li, Z. Ren, *Lactococcus cremoris* D2022 alleviates hyperuricemia and suppresses renal inflammation via potential gut-kidney axis., *Food & Function* (2024). <https://doi.org/10.1039/d4fo00118d>.
- [56] Y. Li, L. Li, J. Tian, F. Zheng, H. Liao, Z. Zhao, Y. Chen, J. Pang, T. Wu, Insoluble fiber in barley leaf attenuates hyperuricemic nephropathy by modulating gut microbiota and short-chain fatty acids, *Foods* 11 (2022) 3482. <https://doi.org/10.3390/foods11213482>.
- [57] L. Zeng, Y. Deng, Q. He, K. Yang, J. Li, W. Xiang, H. Liu, X. Zhu, H. Chen, Safety and efficacy of probiotic supplementation in 8 types of inflammatory arthritis: A systematic review and meta-analysis of 34 randomized controlled trials, *Frontiers in Immunology* 13 (2022). <https://doi.org/10.3389/fimmu.2022.961325>.
- [58] F. Martinon, Mechanisms of uric acid crystal-mediated autoinflammation, *Immunological Reviews* 233 (2010) 218–232. <https://doi.org/10.1111/j.0105-2896.2009.00860.x>.
- [59] Q. Lv, D. Xu, X. Zhang, X. Yang, P. Zhao, X. Cui, X. Liu, W. Yang, G. Yang, S. Xing, Association of hyperuricemia with immune disorders and intestinal barrier dysfunction, *Front. Physiol.* 11 (2020) 524236. <https://doi.org/10.3389/fphys.2020.524236>.

- [60] Y. Guo, H. Li, Z. Liu, C. Li, Y. Chen, C. Jiang, Y. Yu, Z. Tian, Impaired intestinal barrier function in a mouse model of hyperuricemia, *Mol Med Report* (2019). <https://doi.org/10.3892/mmr.2019.10586>.
- [61] D. Xu, Q. Lv, X. Wang, X. Cui, P. Zhao, X. Yang, X. Liu, W. Yang, G. Yang, G. Wang, P. Wang, Z. Wang, Z. Li, S. Xing, Hyperuricemia is associated with impaired intestinal permeability in mice, *American Journal of Physiology-Gastrointestinal and Liver Physiology* 317 (2019) G484–G492. <https://doi.org/10.1152/ajpgi.00151.2019>.
- [62] Y.-X. Xu, L.-D. Liu, J.-Y. Zhu, S.-S. Zhu, B.-Q. Ye, J.-L. Yang, J.-Y. Huang, Z.-H. Huang, Y. You, W.-K. Li, J.-L. He, M. Xia, Y. Liu, *Alistipes indistinctus*-derived hippuric acid promotes intestinal urate excretion to alleviate hyperuricemia, *Cell Host & Microbe* 32 (2024) 366–381.e9. <https://doi.org/10.1016/j.chom.2024.02.001>.
- [63] X. Zhao, F. Peng, Z. Liu, Z. Peng, Q. Guan, P. Cai, S. Xiong, Q. Yu, M. Xie, T. Xiong, Lactic acid bacteria with anti-hyperuricemia ability: Screening *in vitro* and evaluating in mice, *Food Bioscience* 52 (2023) 102411. <https://doi.org/10.1016/j.fbio.2023.102411>.
- [64] N. Qin, M. Qin, W. Shi, L. Kong, L. Wang, G. Xu, Y. Guo, J. Zhang, Q. Ma, Investigation of pathogenesis of hyperuricemia based on untargeted and targeted metabolomics, *Sci Rep* 12 (2022). <https://doi.org/10.1038/s41598-022-18361-y>.
- [65] N. Qin, Y. Jiang, W. Shi, L. Wang, L. Kong, C. Wang, Y. Guo, J. Zhang, Q. Ma, High-throughput untargeted serum metabolomics analysis of hyperuricemia patients by UPLC-Q-TOF/MS, *Evidence-Based Complementary and Alternative Medicine* 2021 (2021) 1–15. <https://doi.org/10.1155/2021/5524772>.
- [66] X. Zhao, P. Cai, S. Xiong, B. Wei, T. Du, T. Huang, Q. Yu, M. Xie, T. Xiong, *Lactacaseibacillus rhamnosus* NCUH061012 alleviates hyperuricemia via modulating gut microbiota and intestinal metabolites in mice, *Food Bioscience* 58 (2024) 103699. <https://doi.org/10.1016/j.fbio.2024.103699>.
- [67] H. Liu, R. Xie, Q. Dai, J. Fang, Y. Xu, B. Li, Exploring the mechanism underlying hyperuricemia using comprehensive research on multi-omics, *Sci Rep* 13 (2023). <https://doi.org/10.1038/s41598-023-34426-y>.
- [68] X. Shen, C. Wang, N. Liang, Z. Liu, X. Li, Z. Zhu, T.R. Merriman, N. Dalbeth, R. Terkeltaub, C. Li, H. Yin, Serum metabolomics identifies dysregulated pathways and potential metabolic biomarkers for hyperuricemia and gout, *Arthritis & Rheumatology* 73 (2021) 1738–1748. <https://doi.org/10.1002/art.41733>.
- [69] J. Li, L. Zhang, T. Wu, Y. Li, X. Zhou, Z. Ruan, Indole-3-propionic acid improved the intestinal barrier by enhancing epithelial barrier and mucus barrier, *J. Agric. Food Chem.* 69 (2021) 1487–1495. <https://doi.org/10.1021/acs.jafc.0c05205>.
- [70] S.A. Scott, J. Fu, P.V. Chang, Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor, *Proc. Natl. Acad. Sci. U.S.A.* 117 (2020) 19376–19387. <https://doi.org/10.1073/pnas.2000047117>.
- [71] S. Estrach, C.-M. Vivier, C.C. Féral, ECM and epithelial stem cells: The scaffold of destiny, *Front. Cell Dev. Biol.* 12 (2024) 1359585. <https://doi.org/10.3389/fcell.2024.1359585>.
- [72] S. Pompili, G. Latella, E. Gaudio, R. Sferra, A. Vetuschi, The charming world of the extracellular matrix: A dynamic and protective network of the intestinal wall, *Front. Med.* 8 (2021) 610189. <https://doi.org/10.3389/fmed.2021.610189>.
- [73] A. Vilaridi, S. Przyborski, C. Mobbs, A. Rufini, C. Tufarelli, Current understanding of the interplay between extracellular matrix remodelling and gut permeability in health and disease, *Cell Death Discov.* 10 (2024) 258. <https://doi.org/10.1038/s41420-024-02015-1>.
- [74] Y.D. Benoit, J.-F. Groulx, D. Gagné, J.-F. Beaulieu, RGD-dependent epithelial cell-matrix interactions in the human intestinal crypt, *Journal of Signal Transduction* 2012 (2012) 1–10. <https://doi.org/10.1155/2012/248759>.
- [75] C. Knudsen, A. Moriya, E. Nakato, R. Gulati, T. Akiyama, H. Nakato, Chondroitin sulfate regulates proliferation of drosophila intestinal stem cells, *PLoS Genet* 21 (2025) e1011686. <https://doi.org/10.1371/journal.pgen.1011686>.
- [76] B. Stockinger, K. Shah, E. Wincent, AHR in the intestinal microenvironment: Safeguarding barrier function, *Nat Rev Gastroenterol Hepatol* 18 (2021) 559–570. <https://doi.org/10.1038/s41575-021-00430-8>.
- [77] C. Schiering, E. Wincent, A. Metidji, A. Iseppon, Y. Li, A.J. Potocnik, S. Omenetti, C.J. Henderson, C.R. Wolf, D.W. Nebert, B. Stockinger, Feedback control of AHR signalling regulates intestinal immunity, *Nature* 542 (2017) 242–245. <https://doi.org/10.1038/nature21080>.
- [78] C. Lei, R. Sun, G. Xu, Y. Tan, W. Feng, C.J. McClain, Z. Deng, Enteric VIP-producing neurons maintain gut microbiota homeostasis through regulating epithelium fucosylation, *Cell Host & Microbe* 30 (2022) 1417–1434.e8. <https://doi.org/10.1016/j.chom.2022.09.001>.

- [79] L. Van Baarle, M. Stakenborg, G. Matteoli, Enteric neuro-immune interactions in intestinal health and disease, *Seminars in Immunology* 70 (2023) 101819. <https://doi.org/10.1016/j.smim.2023.101819>.
- [80] Y. Li, H. Li, R. Wang, Y. Yu, X. Liu, Z. Tian, Protective effect of sodium butyrate on intestinal barrier damage and uric acid reduction in hyperuricemia mice, *Biomedicine & Pharmacotherapy* 161 (2023) 114568. <https://doi.org/10.1016/j.biopha.2023.114568>.
- [81] X. Zhou, S. Ji, L. Chen, X. Liu, Y. Deng, Y. You, M. Wang, Q. He, B. Peng, Y. Yang, X. Chen, H.Y. Kwan, L. Zhou, J. Chen, X. Zhao, Gut microbiota dysbiosis in hyperuricaemia promotes renal injury through the activation of NLRP3 inflammasome, *Microbiome* 12 (2024) 109. <https://doi.org/10.1186/s40168-024-01826-9>.
- [82] Q. Lv, J. Zhou, C. Wang, X. Yang, Y. Han, Q. Zhou, R. Yao, A. Sui, A dynamics association study of gut barrier and microbiota in hyperuricemia, *Front. Microbiol.* 14 (2023) 1287468. <https://doi.org/10.3389/fmicb.2023.1287468>.
- [83] X. Liu, Q. Lv, H. Ren, L. Gao, P. Zhao, X. Yang, G. Yang, D. Xu, G. Wang, W. Yang, P. Wang, Z. Wang, S. Xing, The altered gut microbiota of high-purine-induced hyperuricemia rats and its correlation with hyperuricemia, *PeerJ* 8 (2020) e8664. <https://doi.org/10.7717/peerj.8664>.
- [84] A. Hussain, B. Rui, H. Ullah, P. Dai, K. Ahmad, J. Yuan, Y. Liu, M. Li, *Limosilactobacillus reuteri* HCS02-001 attenuates hyperuricemia through gut microbiota-dependent regulation of uric acid biosynthesis and excretion, *Microorganisms* 12 (2024) 637. <https://doi.org/10.3390/microorganisms12040637>.
- [85] C. Li, M. Tang, X. Li, X. Zhou, Community dynamics in structure and function of honey bee gut bacteria in response to winter dietary shift, *mBio* 13 (2022) e01131-22. <https://doi.org/10.1128/mbio.01131-22>.
- [86] M. Papp, A.G. Tóth, L. Békési, R. Farkas, L. Makrai, G. Maróti, N. Solymosi, Apis mellifera filamentous virus from a honey bee gut microbiome survey in hungary, *Sci Rep* 14 (2024) 5803. <https://doi.org/10.1038/s41598-024-56320-x>.
- [87] M. Furuhashi, New insights into purine metabolism in metabolic diseases: Role of xanthine oxidoreductase activity, *American Journal of Physiology-Endocrinology and Metabolism* 319 (2020) E827–E834. <https://doi.org/10.1152/ajpendo.00378.2020>.
- [88] M.S. Riaz Rajoka, R. Thirumdas, H.M. Mehwish, M. Umair, M. Khurshid, H.F. Hayat, Y. Phimolsiripol, N. Pallarés, F.J. Martí-Quijal, F.J. Barba, Role of food antioxidants in modulating gut microbial communities: Novel understandings in intestinal oxidative stress damage and their impact on host health, *Antioxidants* 10 (2021) 1563. <https://doi.org/10.3390/antiox10101563>.
- [89] J.Y. Noh, N. Farhataziz, M.T. Kinter, X. Yan, Y. Sun, Colonic dysregulation of major metabolic pathways in experimental ulcerative colitis, *Metabolites* 14 (2024) 194. <https://doi.org/10.3390/metabo14040194>.
- [90] J. Hernandez-Baixaui, G. Chomiciute, H. Tracey, I. Mora, A.J. Cortés-Espinar, J. Ávila-Román, N. Abasolo, H. Palacios-Jordan, E. Foguet-Romero, D. Suñol, M. Galofré, J.M. Alcaide-Hidalgo, L. Baselga-Escudero, J.M. Del Bas, M. Mulero, Exploring metabolic and gut microbiome responses to paraquat administration in male wistar rats: Implications for oxidative stress, *Antioxidants* 13 (2024) 67. <https://doi.org/10.3390/antiox13010067>.
- [91] E. Yang, E. Amenyogbe, J. Zhang, W. Wang, J. Huang, G. Chen, Integrated transcriptomics and metabolomics analysis of the intestine of cobia (*rachycentron canadum*) under hypoxia stress, *Aquaculture Reports* 25 (2022) 101261. <https://doi.org/10.1016/j.aqrep.2022.101261>.
- [92] J. Hurbain, Q. Thommen, F. Anquez, B. Pfeuty, Quantitative modeling of pentose phosphate pathway response to oxidative stress reveals a cooperative regulatory strategy, (2022). <https://doi.org/10.1101/2022.02.04.478659>.
- [93] P.I. Nikel, T. Fuhrer, M. Chavarría, A. Sánchez-Pascuala, U. Sauer, V. De Lorenzo, Reconfiguration of metabolic fluxes in *pseudomonas putida* as a response to sub-lethal oxidative stress, *The ISME Journal* 15 (2021) 1751–1766. <https://doi.org/10.1038/s41396-020-00884-9>.
- [94] S.J. Moon, W. Dong, G.N. Stephanopoulos, H.D. Sikes, Oxidative pentose phosphate pathway and glucose anaplerosis support maintenance of mitochondrial NADPH pool under mitochondrial oxidative stress, *Bioengineering & Transla Med* 5 (2020) e10184. <https://doi.org/10.1002/btm2.10184>.
- [95] E.C. Britt, J. Lika, M.A. Giese, T.J. Schoen, G.L. Seim, Z. Huang, P.Y. Lee, A. Huttenlocher, J. Fan, Switching to the cyclic pentose phosphate pathway powers the oxidative burst in activated neutrophils, *Nat Metab* 4 (2022) 389–403. <https://doi.org/10.1038/s42255-022-00550-8>.

- [96] A. Krüger, N.-M. Grüning, M.M.C. Wamelink, M. Kerick, A. Kirpy, D. Parkhomchuk, K. Bluemlein, M.-R. Schweiger, A. Soldatov, H. Lehrach, C. Jakobs, M. Ralser, The pentose phosphate pathway is a metabolic redox sensor and regulates transcription during the antioxidant response, *Antioxidants & Redox Signaling* 15 (2011) 311–324. <https://doi.org/10.1089/ars.2010.3797>.
- [97] G.A. Martínez-Nava, E. Altamirano-Molina, J. Vázquez-Mellado, C.S. Casimiro-Soriguer, J. Dopazo, C. Lozada-Pérez, B. Herrera-López, L.E. Martínez-Gómez, C. Martínez-Armenta, D.L. Guido-Gómora, S. Valle-Gutiérrez, C. Suarez-Ahedo, M.D.C. Camacho-Rea, M. Martínez-García, G. Gutiérrez-Esparza, L.M. Amezcua-Guerra, Y. Zamudio-Cuevas, K. Martínez-Flores, J. Fernández-Torres, A.I. Burguete-García, Y.C. Orbe-Orihuela, A. Lagunas-Martínez, E.O. Méndez-Salazar, A. Francisco-Balderas, B. Palacios-González, C. Pineda, A. López-Reyes, Metatranscriptomic analysis reveals gut microbiome bacterial genes in pyruvate and amino acid metabolism associated with hyperuricemia and gout in humans, *Sci Rep* 15 (2025) 9981. <https://doi.org/10.1038/s41598-025-93899-1>.
- [98] X. Sun, J. Wen, B. Guan, J. Li, J. Luo, J. Li, M. Wei, H. Qiu, Folic acid and zinc improve hyperuricemia by altering the gut microbiota of rats with high-purine diet-induced hyperuricemia, *Front. Microbiol.* 13 (2022) 907952. <https://doi.org/10.3389/fmicb.2022.907952>.
- [99] S. Song, Y. Lou, Y. Mao, X. Wen, M. Fan, Z. He, Y. Shen, C. Wen, T. Shao, Alteration of gut microbiome and correlated amino acid metabolism contribute to hyperuricemia and Th17-driven inflammation in uox-KO mice, *Front. Immunol.* 13 (2022) 804306. <https://doi.org/10.3389/fimmu.2022.804306>.
- [100] Z. Zhao, Z. Li, Y. Xu, S. Zhao, Q. Fan, Z. Zheng, Energy insufficiency induced by high purine diet: Catalysts for renal impairment in hyperuricemia nephropathy rat model, *Current Research in Food Science* 9 (2024) 100864. <https://doi.org/10.1016/j.crfs.2024.100864>.
- [101] M.M.A. Whitten, C.J. Coates, Re-evaluation of insect melanogenesis research: Views from the dark side, *Pigment Cell Melanoma Res* 30 (2017) 386–401. <https://doi.org/10.1111/pcmr.12590>.
- [102] E.V.S. Motta, J.E. Powell, N.A. Moran, Glyphosate induces immune dysregulation in honey bees, *Anim Microbiome* 4 (2022) 16. <https://doi.org/10.1186/s42523-022-00165-0>.
- [103] F. Li, X. Chen, X. Xu, L. Wang, J. Yan, Y. Yu, X. Shan, R. Zhang, H. Xing, T. Zhang, S. Pan, Alterations of intestinal mucosal barrier, cecal microbiota diversity, composition, and metabolites of yellow-feathered broilers under chronic corticosterone-induced stress: A possible mechanism underlying the anti-growth performance and glycolipid metabolism disorder, *Microbiol Spectr* 12 (2024) e03473-23. <https://doi.org/10.1128/spectrum.03473-23>.