



OPEN ACCESS

EDITED BY

Sandra G. Velleman,
The Ohio State University, United States

REVIEWED BY

Colin Guy Scanes,
University of Wisconsin–Milwaukee,
United States
Xubin Du,
Nanjing Agricultural University, China

*CORRESPONDENCE

Kahina Boukherroub,
✉ kahina@umn.edu

RECEIVED 11 September 2025

REVISED 17 October 2025

ACCEPTED 27 October 2025

PUBLISHED 17 November 2025

CITATION

Nabthonglang J, Gomez A, Rutschke S,
Santativongchai P, Kosonsiriluk S, Studniski M,
Wileman B, Navanukraw C and
Boukherroub K (2025) Effect of bardoxolone
methyl on the lower reproductive tract
microbiome in turkey breeder hens.
Front. Physiol. 16:1703742.
doi: 10.3389/fphys.2025.1703742

COPYRIGHT

© 2025 Nabthonglang, Gomez, Rutschke,
Santativongchai, Kosonsiriluk, Studniski,
Wileman, Navanukraw and Boukherroub. This
is an open-access article distributed under
the terms of the [Creative Commons
Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that
the original publication in this journal is cited,
in accordance with accepted academic
practice. No use, distribution or reproduction
is permitted which does not comply with
these terms.

Effect of bardoxolone methyl on the lower reproductive tract microbiome in turkey breeder hens

Juthamas Nabthonglang^{1,2}, Andres Gomez¹,
Stephanie Rutschke¹, Pitchaya Santativongchai¹,
Sunantha Kosonsiriluk¹, Marissa Studniski³, Ben Wileman³,
Chainarong Navanukraw² and Kahina Boukherroub^{1*}

¹Department of Animal Science, University of Minnesota, St. Paul, MN, United States, ²Department of Animal Science, Faculty of Agriculture, Khon Kaen University, Khon Kaen, Thailand, ³Select Genetics, Willmar, MN, United States

Introduction: Fertility decline in aging turkey breeder hens is associated with reduced sperm storage in the uterovaginal junction (UVJ), inflammation, oxidative stress, and tissue aging. The mucosal microbiome is an important contributor to subfertility, with shifts in immune function, inflammation, and oxidative stress linked to microbial changes. Bardoxolone methyl, a potent activator of the nuclear erythroid 2-related factor 2 (NRF2) pathway, enhances antioxidant defenses and reduces inflammation. This study investigated if bardoxolone methyl treatment alters the microbial composition and diversity of the UVJ and vagina in turkey hens.

Methods: Forty turkey hens (59 weeks old) were randomly assigned to a bardoxolone methyl group (n = 20) or a control group (n = 20). Birds received intramuscular tail injections of bardoxolone methyl or vehicle, every other day for two weeks. Swabs from the UVJ and vagina (VAG) were collected for 16S rRNA sequencing. Microbial diversity, differential taxonomic composition, and predicted functional pathways were assessed using QIIME2, PICRUST2, and R-based statistical packages. Microbiome profiles revealed significant differences between UVJ and VAG communities.

Results: The VAG showed higher bacterial richness, while both sites were dominated by *Firmicutes*, *Proteobacteria*, *Thermoproteota*, and *Actinobacteriota* phyla, indicator species analyses identified enrichment of *Staphylococcus* and *Escherichia* in UVJ, and Lactobacillaceae in VAG. Bardoxolone methyl did not significantly alter global alpha diversity but selectively modulated unweighted beta diversity and low-abundance taxa, enriching *Corynebacterium* in UVJ and rare taxa like *Armatimonadota* and *Omnitrophota* in the VAG. Functional predictions indicated bardoxolone methyl's association with enrichment of pathways including energy metabolism, nucleotide biosynthesis, protein quality control, and redox balance, particularly in the UVJ.

Discussion: This study provides the first characterization of the turkey lower reproductive tract microbiome, revealing tissue-specific communities and functional profiles between the UVJ and vagina. Bardoxolone methyl treatment did not alter overall microbial diversity, but selectively enriched low-abundance taxa and metabolic pathways related to energy metabolism, nucleotide

biosynthesis, and stress resilience, particularly in the UVJ. These findings indicate that bardoxolone methyl treatment can finetune microbial functional capacity without destabilizing overall community structure. The results also highlight the importance of considering tissue-specific differences and functional potential when investigating reproductive function.

KEYWORDS

microbiome, uterovaginal junction, vagina, bardoxolone methyl, turkey

1 Introduction

The United States has the largest turkey industry globally, with turkey meat ranking as the second most consumed poultry meat within the country (Food and Agriculture Organization of the United Nations, 2023). Commercial turkey production depends on breeder hens to produce fertilized eggs, which are subsequently hatched and reared for meat production. However, fertility in turkey breeder hens naturally declines at the later stages of the laying cycle, typically leading to reduced hatchability and productivity in later stages of the laying cycle. Therefore, improving or sustaining reproductive performance in aging breeder hens is critical to optimize flock productivity and maintain economic viability.

The primary factor contributing to fertility decline in aging turkey breeders is their reduced ability to retain sperm within sperm storage tubules (SSTs), specialized tubular structures located at the uterovaginal junction (UVJ) of the reproductive tract (Christensen, 1981; Sasanami et al., 2013). Research indicates that this decline is associated with dysregulation in the sperm selection and storage processes within the vagina and UVJ, respectively. Changes in the immune cell composition and localization, increased inflammation, and accelerated tissue aging have been observed towards the end of laying cycle, when fertility is typically at its lowest (Kosonsiriluk et al., 2023; Chen et al., 2024). These processes are known to increase the production of reactive oxygen species (ROS), leading to oxidative stress within tissues, while simultaneously reducing the intrinsic antioxidant capacity of the cells (Khansari et al., 2009; Biswas, 2016). Encouragingly, reducing oxidative stress and increasing the antioxidant capacity of cells within reproductive tissues is achievable through the use of readily available, naturally derived compounds that activate the Nuclear Factor Erythroid 2-related Factor 2 (NRF2) pathway, such as bardoxolone methyl (Ruiz et al., 2013; Pei et al., 2019; Robledinos-Antón et al., 2019).

Changes in the immune system, inflammation, and oxidative stress have also been associated with changes in the microbiome (Kim et al., 2006; Witkin et al., 2007; Marciano and Vajro, 2017). Recent studies have shown that gut epithelial cells rapidly produce ROS in response to enteric commensal bacteria (M Jones et al., 2012), and similar ROS responses occur in other cell types exposed to microbial signals (O'Connell et al., 2005). These physiologically generated ROS act as second messengers in signaling pathways triggered by proinflammatory cytokines and growth factors, thereby modulating cellular activity (Witkin et al., 2007; Marciano and Vajro, 2017).

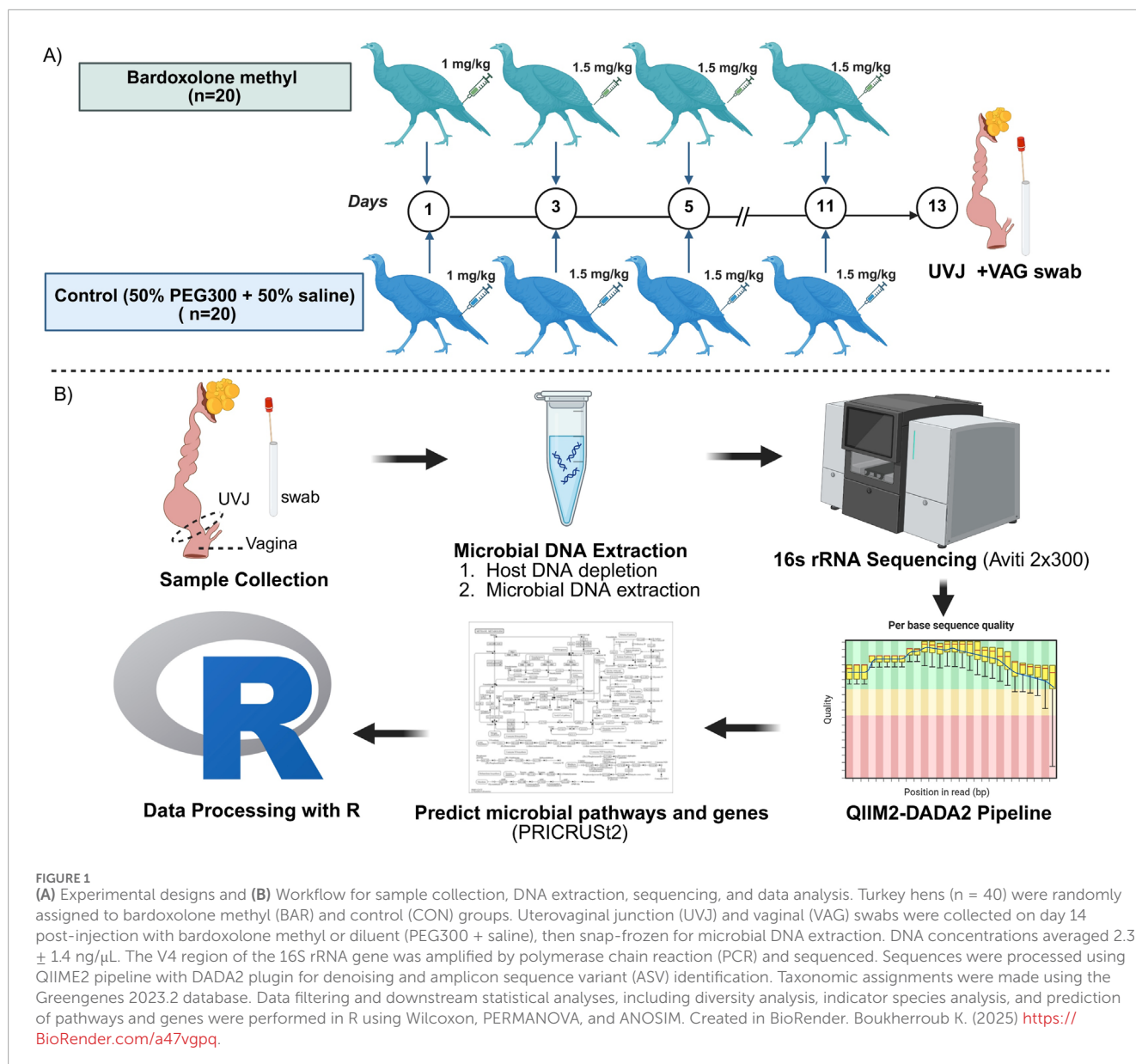
The reproductive tract microbiome has also been implicated in subfertility across multiple species including humans, mice, cows, swine, and ewes (García-Velasco et al., 2017; Deng et al., 2019; Chen et al., 2020; Sanglard et al., 2020). By contrast, no published studies have examined the relationship between microbiome and fertility in avian species. Among the limited research on avian reproductive tract microbiomes, only one study has specifically investigated the vaginal microbiome in chickens, and none have characterized the microbial composition of the turkey UVJ or vagina (Lee et al., 2019; Shterzer et al., 2020; Wen et al., 2021; Kursu et al., 2022). Furthermore, to our knowledge, no studies have attempted to alter the microbial composition of the lower reproductive tract in avian species.

The objective of the present study was to determine if administration of a NRF2-activating compound to turkey hens alters the microbial composition and diversity of the UVJ and vagina. This study provides the first characterization of the vaginal and UVJ microbiome in turkeys, both with and without antioxidant treatment, as well as the associated changes in microbial composition and pathway responses to the treatment.

2 Materials and methods

2.1 Experimental design

A total of 40 turkey breeder hens (*Meleagris gallopavo*), aged 59 weeks (28th week of lay) at the end of the production cycle were kindly donated by a Minnesota turkey breeding company. The hens were housed under standard commercial conditions with *ad libitum* access to a conventional diet containing 18% protein and water. All animal care procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Minnesota under protocol number 2401-41696A. Hens were randomly assigned to two groups: bardoxolone methyl (BAR; n = 20) and control (CON; n = 20). The BAR group received an initial intramuscular injection of 1 mg/kg bardoxolone methyl (HY-13324, purity ≥98%, MedChemExpress LLC., NJ, United States), dissolved in the diluent (50% polyethylene glycol 300 (PEG300) and 50% saline), administered in the tail area. Subsequent injections of 1.5 mg/kg bardoxolone methyl in the diluent were given every other day for 2 weeks. The CON group received equal volumes of the diluent without bardoxolone methyl, following the same injection schedule (Figure 1A). The bardoxolone methyl dose was derived from allometric scaling of the human therapeutic range (0.3–2 mg/kg/day (Nangaku et al., 2020). Based on the average body weight of turkey hens (30–35 lb;



~14.5 kg), this corresponded to an equivalent exposure of 0.9–5.96 mg/kg per injection. The initial low dose was used to evaluate potential local immune or inflammatory reactions; the site was monitored closely, and dosing adjustments were made in consultation with the attending veterinarian. Because turkeys are particularly sensitive to pharmacologic interventions, the final regimen was maintained within the low-to-moderate range of the scaled therapeutic window to balance safety and efficacy, promoting sustained NRF2 activation while minimizing adverse effects.

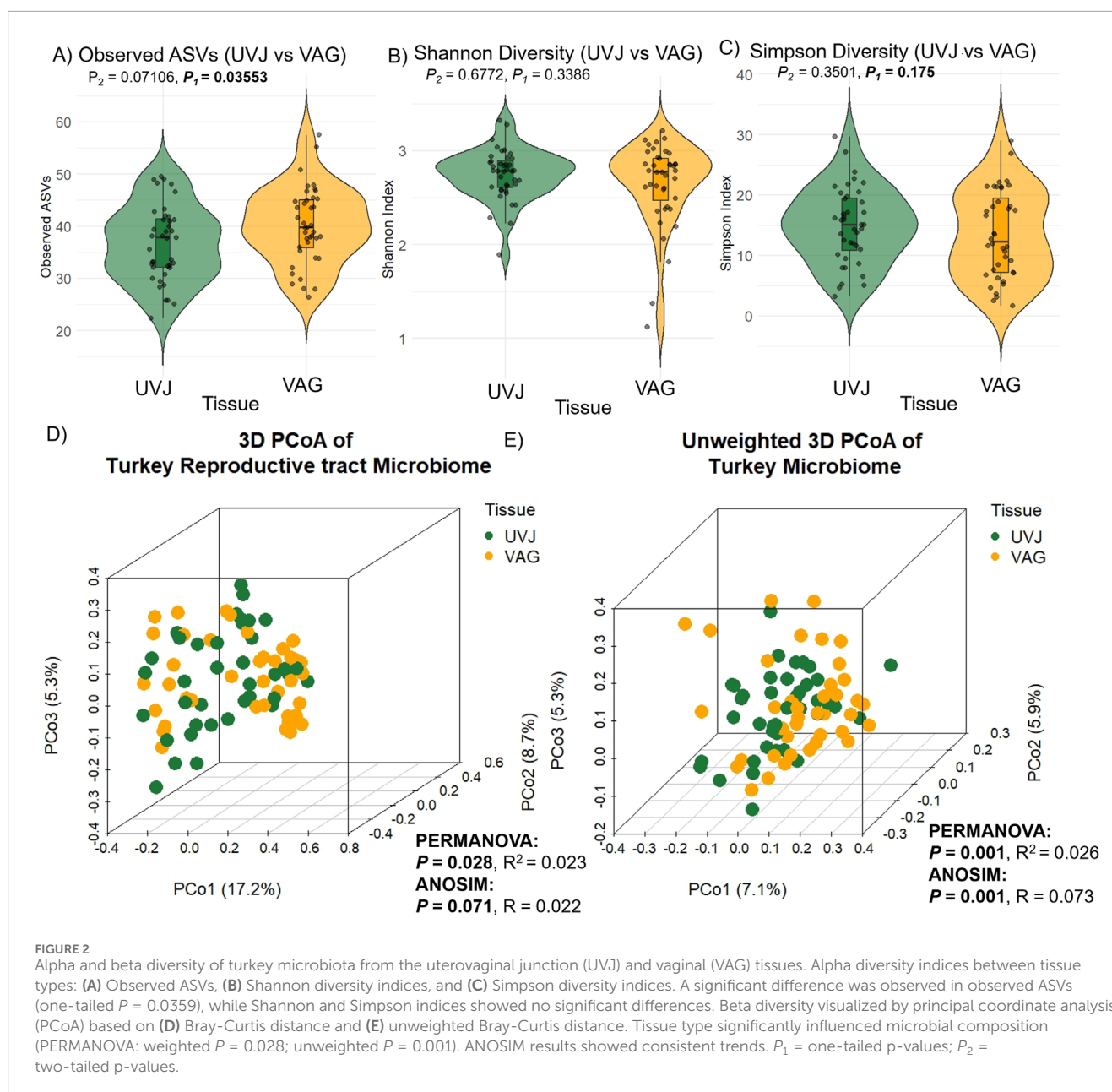
2.2 Sample collection

Following a 2-week treatment period, hens were humanely euthanized using assisted mechanical cervical dislocation in accordance with approved animal care protocols. The lower

reproductive tract was excised by dissection from the uterus to the cloacal opening. Vaginal tissue was sampled from the mid-region of the vagina 1 cm distal to the UVJ. Subsequently, the mucosal surfaces of the excised 1 x 1.5 cm UVJ and vagina (VAG) sections were swabbed using sterile swabs. Swabs were immediately transferred into sterile microcentrifuge tubes and stored at -80°C until DNA extraction and microbial analysis (Figure 1B).

2.3 DNA extraction and 16s rRNA gene sequencing

Microbial DNA was extracted from the UVJ and VAG tissue swabs using the HostZERO™ Microbial DNA Kit (Zymo Research, California, United States), following the manufacturer's instructions to reduce host DNA contamination. The extracted



DNA concentrations were 2.4 ± 1.4 ng/ μ L across samples as measured using a NanoDrop One Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The V4 region of the 16S rRNA gene was amplified using primers 515F (GTGYCAGCMGCCGCGGTAA) and 806R (GGACTACNVGGGTWTCTAAT) using the dual index approach (Gohl et al., 2016), and libraries were paired-end sequenced (2×300 bp) on the AVITI platform at the University of Minnesota Genomics Center (UMGC).

Raw reads (obtained after sequencing) were subjected to quality control using FASTQC (Andrews et al., 2010) and Cutadapt, which was used to trim adapter, primer sequences, and low quality bases (Martin, 2011). Sequences, that were too short were filtered out. High quality sequences were processed using QIIME2 version 2023.2 (Bolyen et al., 2019) and amplicon sequence variants (ASVs)

were determined with the DADA2 plug in using the denoise-paired method (Shterzer et al., 2024; Callahan et al., 2016). Greengenes2 (version 2022.10) was used as the reference database for the taxonomic assignment of ASVs. Potential contaminants were removed using the Decontam package (Callahan and Davis, 2025). ASVs with fewer than 5 total reads across the entire dataset and those present in fewer than 3 samples were filtered out using the labdsv package (Roberts, 2019). Functional predictions of 16S rRNA data was performed via PICRUSt 2 (Langille et al., 2013).

2.4 Statistical analysis

Further analyses including alpha diversity metrics (observed ASVs, Shannon, and Simpson diversity index), beta diversity

TABLE 1 Analysis of variance (ANOVA) for alpha diversity indices (Observed species, Shannon, and Simpson) and beta diversity (Weighted and Unweighted Bray-Curtis) in turkey samples.

Diversity index	Source	P-value
Alpha diversity		
- Observed ASVs	Treatment	0.867
	Tissue	0.045 ^a
	Treatment × Tissue	0.963
- Shannon	Treatment	0.327
	Tissue	0.615
	Treatment × Tissue	0.827
- Simpson	Treatment	0.137
	Tissue	0.330
	Treatment × Tissue	0.974
Beta diversity		
- Weighted Bray-Curtis	Treatment	0.350
	Tissue	0.025 ^a
	Treatment × Tissue	0.984
- Unweighted Bray-Curtis	Treatment	0.140
	Tissue	0.001***
	Treatment × Tissue	0.215

^aSignificant at $P < 0.05$.

(Bray-Curtis dissimilarity), and indicator species analyses were carried out using the R program and the phyloseq (McMurdie and Holmes, 2013), labdsv (Roberts, 2019), ape (Paradis et al., 2019) and vegan (Oksanen et al., 2025) packages.

All graphs were generated using the ggplot2 (Wickham et al., 2025) and pheatmap (Kolde, 2025) packages in R. Bar plots were constructed showing the relative abundance of taxa at the phylum, family, and genus levels. Violin plots were generated to visualize the distribution of indicator species, pathways, and gene abundances across treatment or tissue sites. Heatmaps of the top abundant pathways and genes were also constructed to highlight patterns of functional variation across samples. Pathway and gene prediction of microbial metabolic pathways was performed using PICRUSt2 (Douglas et al., 2020), and pathway-level differences were further analyzed using indicator species analysis with pathway annotation based on MetaCyc and KEGG databases.

Statistical analyses for alpha and beta diversity comparisons, as well as relative abundance comparisons, were primarily performed using the Wilcoxon rank-sum test to identify statistically significant differences in ASV, pathway, and gene abundances across the following comparisons: UVJ vs. VAG regardless of treatment; BAR vs. CON regardless of tissue type; BAR vs. CON within

UVJ; and BAR vs. CON within VAG. Beta diversity analysis was conducted using Bray-Curtis dissimilarity, and group differences were assessed using both permutational multivariate analysis of variance (PERMANOVA) and analysis of similarities (ANOSIM). For variables with normally distributed data as determined by the Shapiro-Wilk test, additional parametric tests such as t-test and analysis of variance (ANOVA) were applied. A P -value < 0.05 was considered statistically significant. Differential abundance analyses were performed using Wilcoxon rank-sum tests. Differences were considered statistically significant at $P < 0.05$.

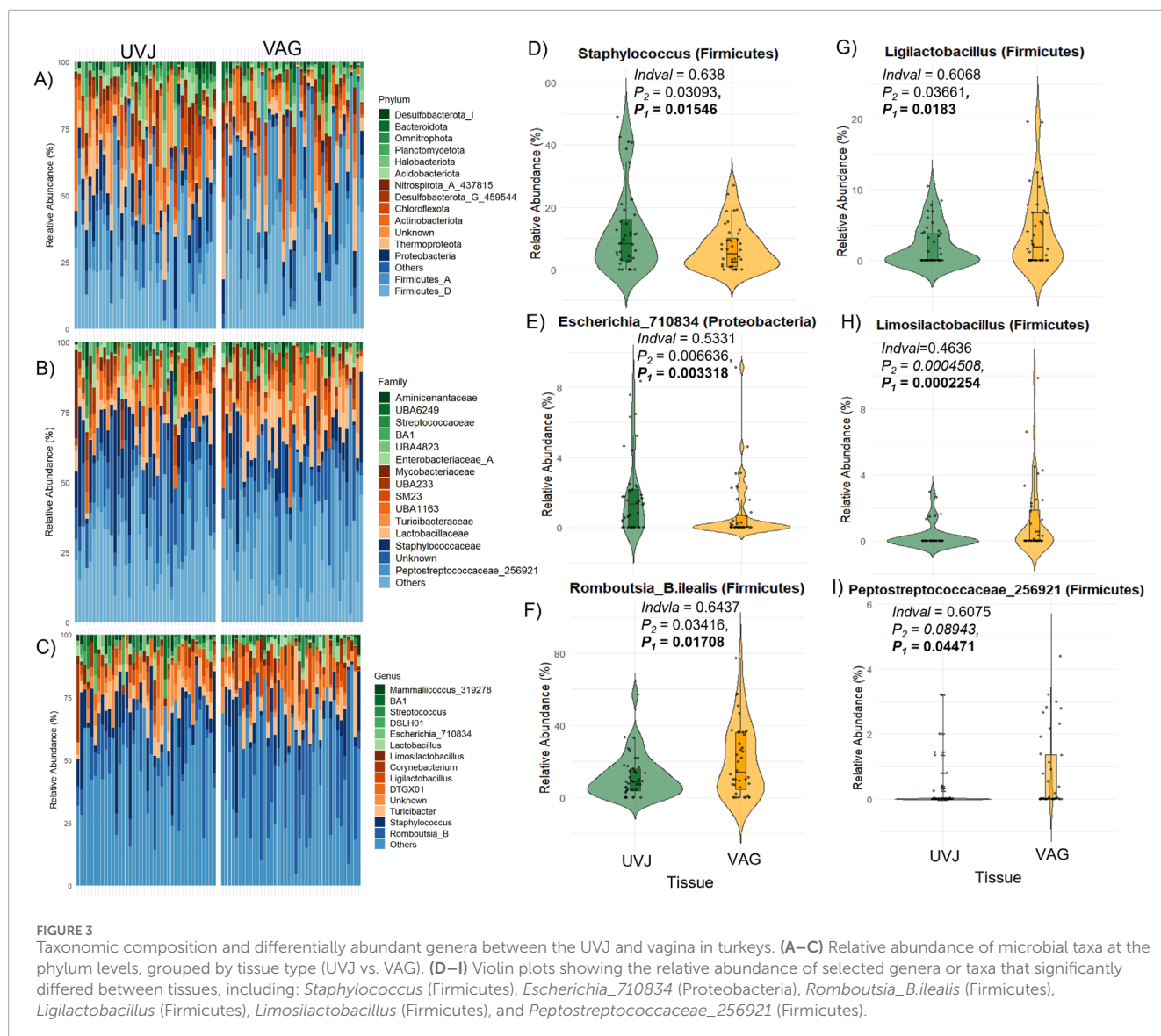
3 Results

3.1 Distinct microbial diversity and taxonomic composition between the UVJ and vagina

Microbial composition analysis revealed significantly higher bacterial richness in the VAG compared to the UVJ irrespective of treatment group as indicated by the number of observed ASVs (Wilcoxon one-tailed test, $P = 0.0359$; Figure 2A). In contrast, the Shannon (which accounts for both richness and evenness) and Simpson diversity indices (which reflects species dominance) showed no significant differences, suggesting comparable community evenness (Figures 2B,C). ANOVA results supported this pattern, confirming a tissue-specific effect on richness ($P = 0.045$) without affecting overall diversity indices (Table 1).

Multivariate analysis based on Bray-Curtis dissimilarity revealed distinct microbial communities between UVJ and VAG, regardless of treatment. PCoA showed significant clustering or separation between tissue types (Figures 2D,E), which was confirmed via PERMANOVA for both weighted ($P = 0.028$; $R^2 = 0.023$) and unweighted ($P = 0.001$; $R^2 = 0.026$) distances (Table 1). The lower P -value and slightly greater R^2 between tissue types in the unweighted analysis may suggest that differences in microbiome composition between UVJ and VAG are driven by low-abundance taxa. ANOSIM further supported compositional dissimilarity between UVJ and VAG microbiomes, with both weighted ($P = 0.071$, $R = 0.022$) and unweighted ANOSIM ($P = 0.001$, $R = 0.073$) indicating minor but significant differences, particularly for the unweighted analysis.

Taxonomic profiling highlighted tissue-specific differences at multiple taxonomic levels. Both sites were dominated by the phyla *Firmicutes*, *Proteobacteria*, *Thermoproteota*, and *Actinobacteriota* (Figures 3A–C), with *Peptostreptococcaceae_256921*, *Staphylococcaceae*, *Lactobacillaceae*, and *Enterobacteriaceae_A* among the most abundant families, and *Mammaliicoccus_319278*, *Streptococcus*, *Lactobacillus*, *Ligilactobacillus*, *Limosilactobacillus*, *Escherichia_710834*, and *Romboutsia_B* as the predominant genera (Supplementary Figures S1A,B). At the ASV level, indicator species analyses revealed that *Staphylococcus* and *Escherichia_710834* were enriched in UVJ samples, while *Romboutsia_B.ilealis*, *Ligilactobacillus*, and *Limosilactobacillus* were more abundant in the VAG (Figures 3D–I). The relative abundances of other discriminatory taxa across tissues are summarized in Table 2, reflecting distinct microbial niches along the turkey reproductive tract.



Taken together, these results indicate that the UVJ and vagina harbor distinct microbial communities not only in terms of diversity but also taxonomic composition, with specific bacterial genera preferentially colonizing each anatomical site.

Additionally, microbiota diversity was compared between BAR and CON groups irrespective of site (UVJ or VAG). No significant differences were observed in alpha diversity (observed ASVs, Shannon index), though Simpson diversity showed a non-significant trend toward lower diversity in the CON group ($P = 0.0797$; **Supplementary Figures S2A–C**). Beta diversity analysis did not show significant group clustering or separation ($P > 0.05$; **Supplementary Figures S2D,E**), suggesting BAR did not broadly alter microbial diversity. However, ASV-level indicator species analysis revealed minor differential enrichment of specific taxa between BAR and CON regardless of tissue. For example, *Bin103.sp002238925* (Spirochaetota) and *Varibaculum* (Actinobacteriota) were higher in BAR, while *Anaerolineales* (Chloroflexota) and *Ligilactobacillus* (Firmicutes) were enriched in CON (Wilcoxon

test, $P < 0.05$; **Supplementary Figures S3**), indicating selective modulation by BAR despite stable overall diversity.

3.2 Predicted microbial pathways and genes differ between UVJ and vaginal microbiomes

Predicted microbial metabolic pathways and specific genes as revealed via PICRUSt2 indicated distinct functional profiles between the UVJ and VAG microbiomes. Overall, the most abundant predicted pathways in UVJ and vagina included the Pentose phosphate (non-oxidative) I, Adenosine *de novo* biosynthesis I (IMP & Asp), and the Calvin-Benson-Bassham cycle (**Figure 4A**), reflecting core microbial metabolic functions. At the gene level, highly represented functions included ribonucleoside-diphosphate reductase, Alanine dehydrogenase, 2-OG dehydrogenase E2, glutamate synthase, and ATP sulfurylase, among others (**Figure 4B**), further emphasizing conserved microbial processes across tissues.

TABLE 2 Relative abundance of discriminatory bacteria across tissue.

Taxa	Abundance in UVJ	Abundance in vagina	Indicator value	Wilcoxon p-value
<i>s__Limosilactobacillus.ingluviei</i> (Firmicutes_D)	0.2421 ± 0.7789	0.9026 ± 1.6968	0.3745	0.00188
<i>s__Limosilactobacillus.fermentum</i> (Firmicutes_D)	0.7926 ± 1.6971	0.2259 ± 0.8241	0.2724	0.01925
<i>g__Anaerococcus</i> (Firmicutes_A)	0.000 ± 0.000	0.4785 ± 1.3771	0.2500	0.00083
<i>g__Clostridium</i> (Firmicutes_A)	0.0134 ± 0.0849	0.1174 ± 0.4067	0.2243	0.00433
<i>g__Enterococcus</i> (Firmicutes_D)	0.0616 ± 0.3894	0.535 ± 2.1398	0.2242	0.01236
<i>s__UTCFX2.sp002050125</i> (Chloroflexota)	0.3207 ± 0.992	0.000 ± 0.000	0.1500	0.04418
<i>f__UBA6902</i> (Nitrospirota)	0.3321 ± 1.5605	0.000 ± 0.000	0.1500	0.01181
<i>f__UBA6249</i> (Omnitrophota)	0.000 ± 1e-04	0.2726 ± 0.8603	0.1500	0.04629
<i>f__Ruminococcaceae</i> (Firmicutes_A)	0.000 ± 0.000	0.0518 ± 0.2025	0.1250	0.02249

Abundance data are reported as the mean ± standard deviation. Numbers in bold show which tissue has higher abundance.

However, indicator species analysis identified several pathways that were significantly enriched in specific tissue types. Notably, dAMP synthesis II (*de novo*), Coenzyme A biosynthesis, and Glycolysis pathways were significantly more abundant in the VAG microbiome ($P < 0.001$ for all; **Figures 4C–E**), suggesting enhanced nucleotide and energy metabolism in this region.

In contrast, UVJ samples showed higher levels of specific predicted genes, such as the sarcosine oxidase gene (*soxG*, subunit gamma), indicating tissue-specific microbial functions related to amino acid metabolism (**Figure 4F**). Additionally, genes involved in ribonucleoside-diphosphate biosynthesis were enriched in VAG samples (**Figure 4G**), while choline dehydrogenase gene (*chdh*) tended to be more abundant in UVJ, though this difference was marginally significant ($P = 0.05$; **Figure 4H**). Together, these findings highlight both shared core metabolic pathways and site-specific gene-level metabolic potentials along the lower reproductive tract.

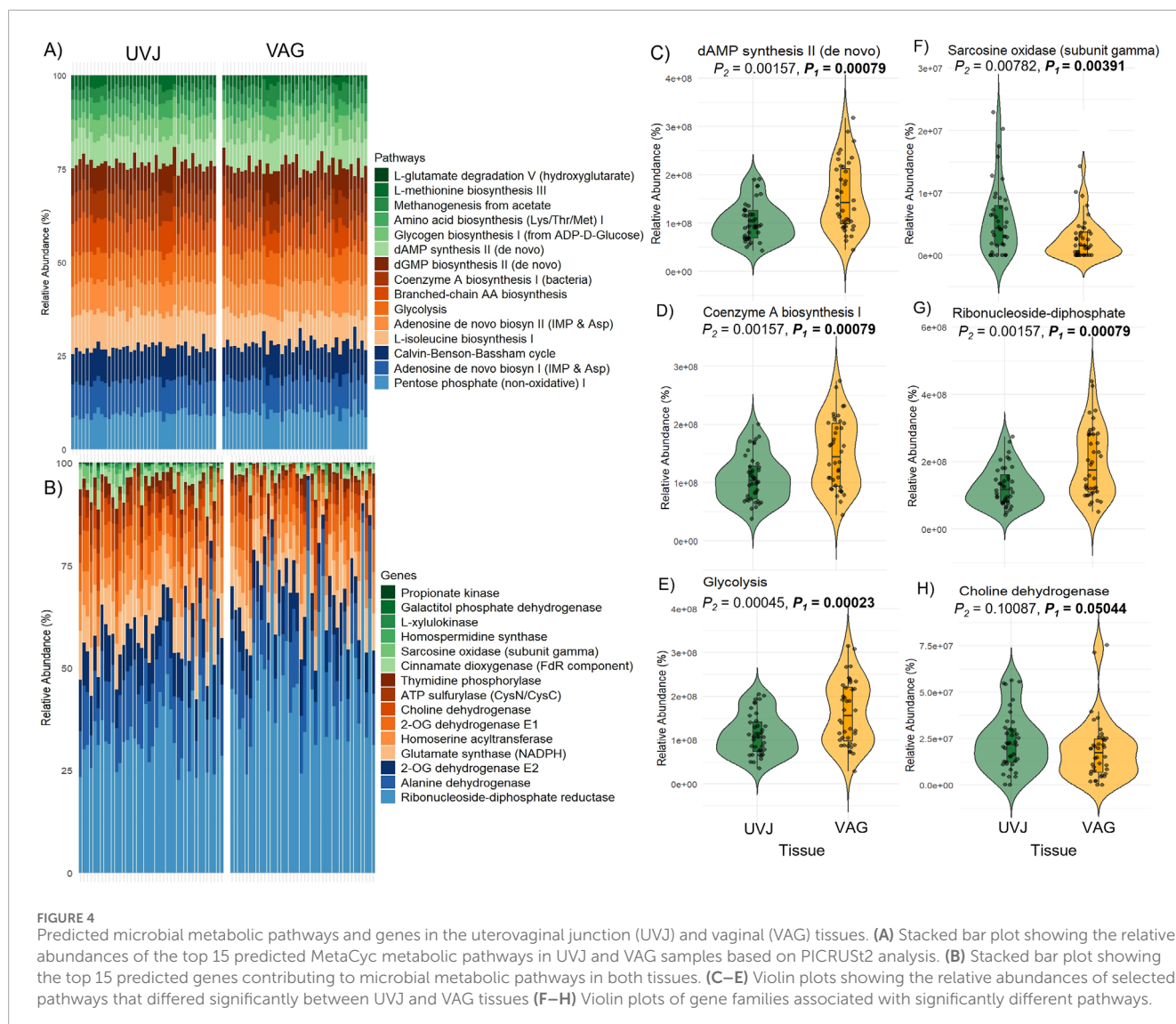
3.3 Impact of bardoxolone methyl on microbial diversity and predicted functions in the UVJ

The effect of bardoxolone methyl on the UVJ microbiome was evaluated through taxonomic and functional analyses. Alpha diversity metrics (observed ASVs, Shannon, and Simpson indices) showed no significant difference between BAR and CON groups, indicating stable species richness and evenness (**Figures 5A–C**). Although weighted distances showed no difference ($P > 0.05$; **Figure 5D**), unweighted beta diversity (Bray–Curtis) analyses revealed modest but significant compositional clustering (PERMANOVA $P = 0.038$, $R^2 = 0.039$; ANOSIM $P = 0.063$, $R = 0.055$), suggesting BAR may influence low-abundance taxa (**Figure 5E**).

Dominant phyla in the UVJ across BAR and CON groups included *Firmicutes*, *Proteobacteria*, *Thermoproteota*, and *Actinobacteriota*. Families such as *Staphylococcaceae* and *Peptostreptococcaceae* were prevalent, with key genera including *Staphylococcus*, *Romboutsia*, *B.ilealis*, *Turicibacter*, *Corynebacterium*, and *Ligilactobacillus* (**Figures 6A–C**). Indicator species analysis identified ASVs from *Corynebacterium* as significantly enriched in BAR, while *Pseudomonadaceae* and *Burkholderia* were more abundant in CON group (**Figures 6D–F**), suggesting selective promotion of potentially beneficial taxa and suppression of potentially pro-inflammatory ones by BAR.

Functional inference analysis was then conducted to identify metabolic consequences associated with these taxonomic changes. Microbial metabolic potential was predicted based on pathway and gene-level analyses. BAR-treated samples exhibited an increased relative abundance of microbial pathways involved in carbohydrate and nucleotide metabolism, including sucrose biosynthesis I, NAD biosynthesis, histidine biosynthesis, nucleotide biosynthesis, and the reductive tricarboxylic acid (TCA) cycle (**Figures 7A,B**). At the gene level, BAR samples exhibited higher abundance of regulatory genes, including Cell cycle kinase A (*CckA*), Chromosomal virulence histidine kinase G (*ChvG*), Chromosomal virulence response regulator I (*ChvI*), Cyclic-di-GMP phosphodiesterase F (*TipF*), which are associated with environmental sensing and microbial interactions (**Figures 7C,D**).

Despite these functional enrichment, overall pathway- and gene-level alpha and beta diversity remained unchanged between BAR and CON groups in the UVJ (**Supplementary Figures S5A–E, S5A–E**), indicating targeted or selected microbial modulation without widespread disruption. These findings suggest that BAR supplementation selectively alters the UVJ microbiome by promoting



a potentially beneficial microbial environment and enhancing functional pathways associated with energy metabolism and environmental adaptability.

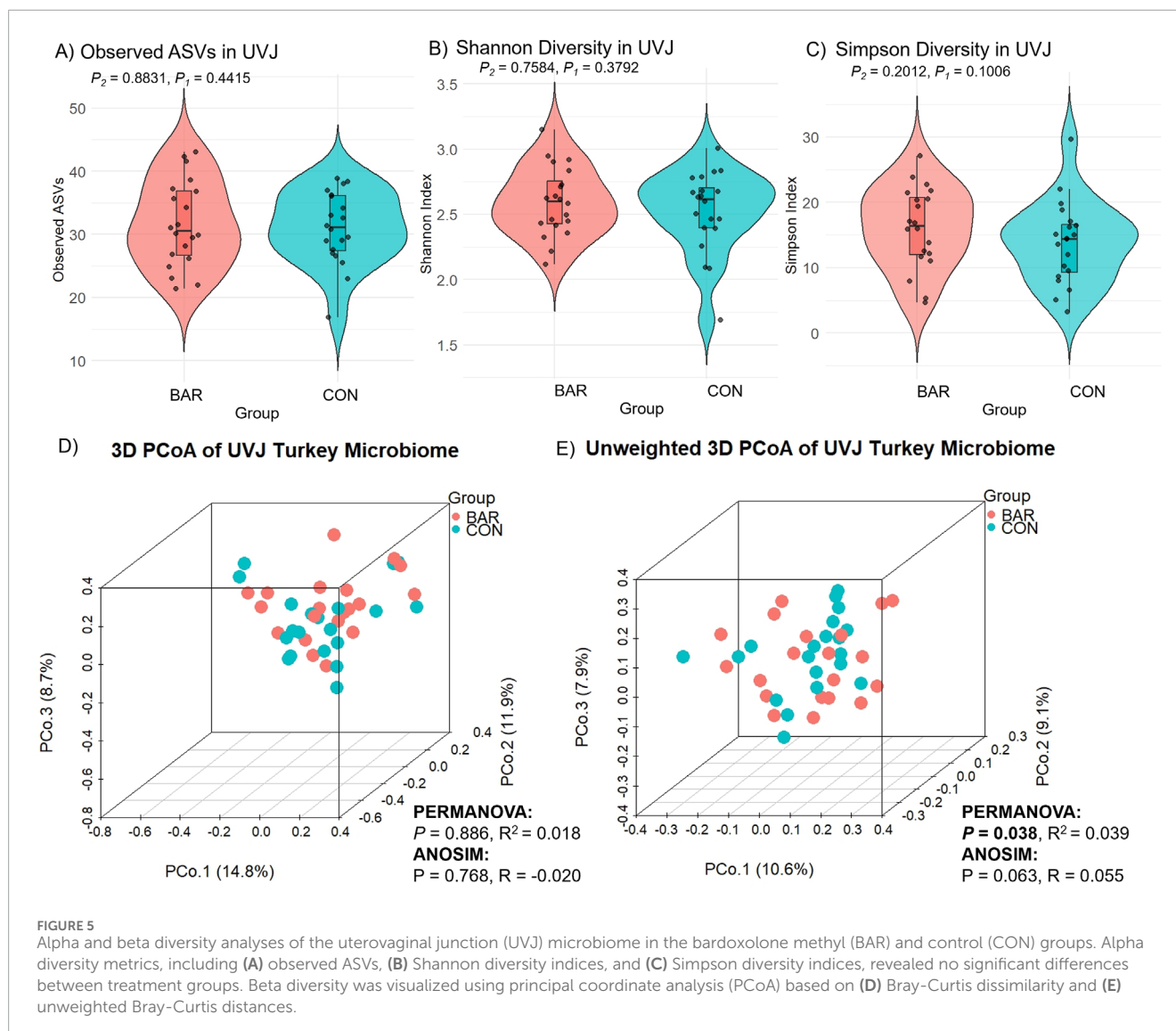
3.4 Impact of bardoxolone methyl on microbial diversity and predicted functions in the vagina

In contrast to the UVJ, BAR treatment had minimal effect on vaginal microbial diversity. Alpha and beta diversity metrics showed no significant differences between groups (Figures 8A–E), indicating compositional stability. Taxonomic profiling revealed *Firmicutes*, *Thermoproteota*, and *Proteobacteria* as dominant phyla in the vagina across both CON and BAR groups (Figure 9A). At the family level, *Peptostreptococcaceae*, *Lactobacillaceae*, and *Staphylococcaceae* were predominant (Figure 9B), with *Limosilactobacillus*, *Ligilactobacillus*, and *Streptococcus* emerging as the most abundant genera (Figure 9C).

However, indicator species analysis identified two low-abundance ASVs significantly enriched in the BAR group: *DTIZ01.sp011370625* (*Armatimonadota*) and *UBA6249* (*Omnitrophota*), both showing higher relative abundance in the vagina of BAR-treated hens compared to CON animals (Figure 9D). These results suggest that BAR selectively modulates specific low-abundance taxa without affecting overall community structure, consistent with patterns observed in the UVJ.

To assess functional implications, predicted microbial metabolic pathways and gene profiles were analyzed. Although overall diversity of KEGG Ortholog pathways and gene functions did not differ significantly between groups (Supplementary Figures S6A–E), deeper analysis revealed distinct functional enrichments in the BAR group.

Specifically, several pathways were enriched, including gluconeogenesis I, polyisoprenoid biosynthesis, coenzyme A biosynthesis, and guanosine nucleotides degradation III (Figure 10A) in the BAR group. These pathways are involved in energy metabolism, nucleotide turnover, and membrane



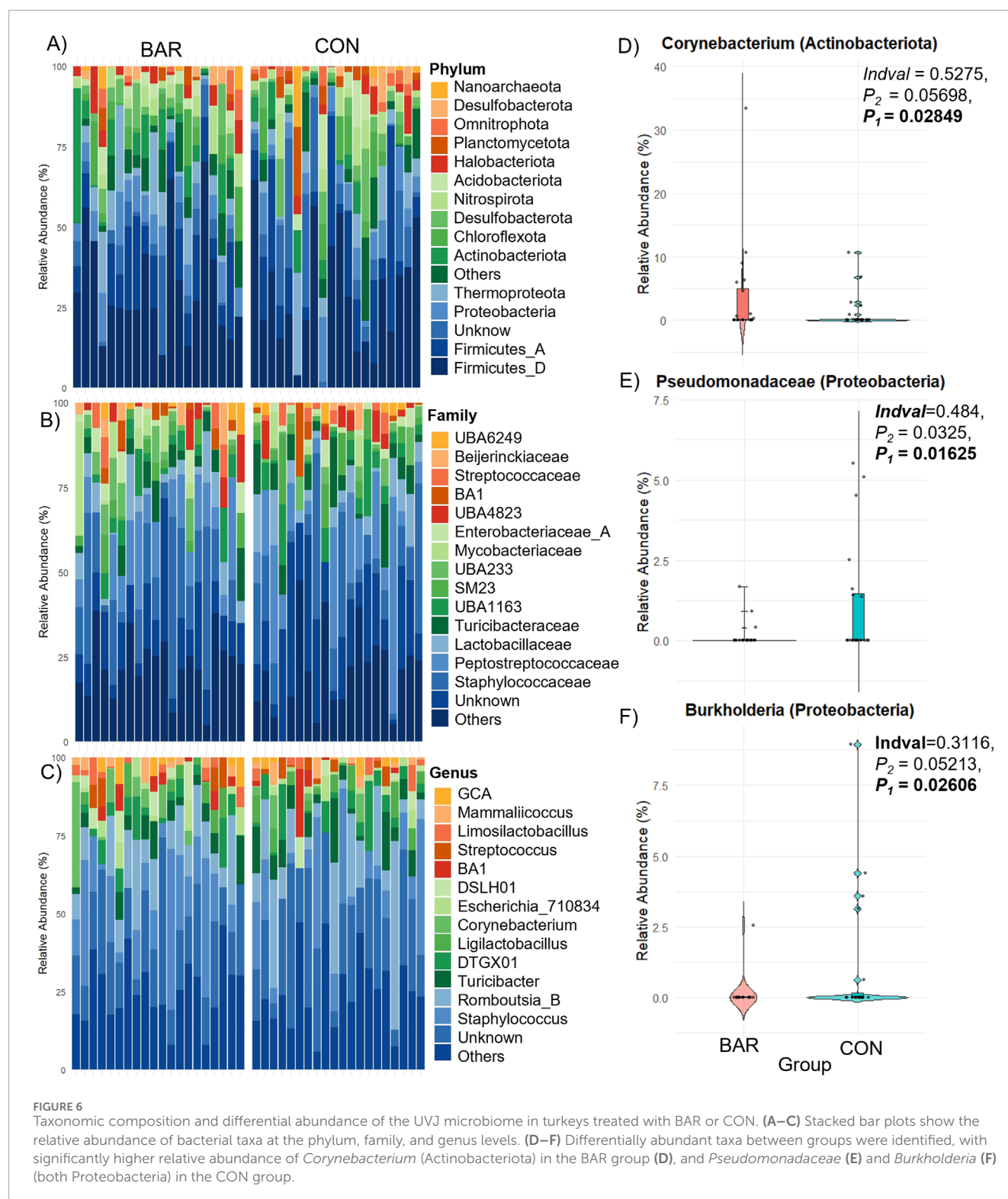
biosynthesis, suggesting enhanced metabolic activity. At the gene level, BAR-treated birds showed increased abundance of genes associated with DNA replication (e.g., *FtsK*), translation (ribosomal protein L30), central carbon metabolism (pyruvate dehydrogenase alpha subunit, *pdhA*), and protein quality control (*ClpC* ATPase) (Figure 10B). Indicator analysis further confirmed enrichment of genes such as pyruvate dehydrogenase (*pdhA* and *pdhB* subunits) and betaine aldehyde dehydrogenase (*betA*) ($P < 0.01$), potentially pointing to improved microbial energy production and stress resilience in the BAR group (Figures 10C,D). Additional enriched functions in BAR treated animals included genes related to membrane transport and redox regulation, such as the indigoidine exporter and menaquinone biosynthesis enzymes (*MenF/MenH*).

4 Discussion

This study provides the first comprehensive characterization of the microbial communities in the lower reproductive tract of turkey

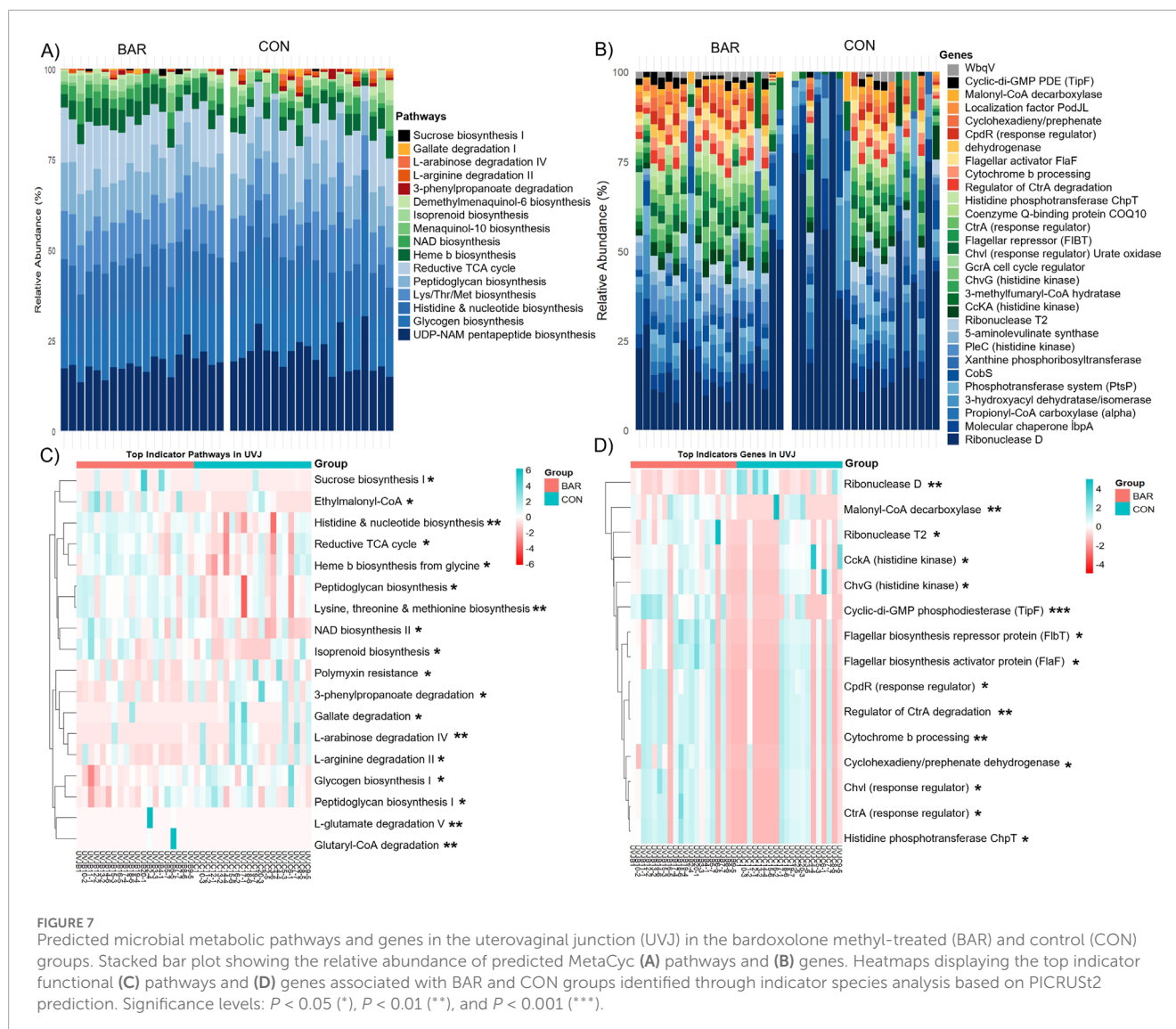
hens, focusing on the vagina and the UVJ, and further evaluates their modulation by the potent NRF2 activator bardoxolone methyl. We found that the microbiome composition and predicted metabolic functions differed markedly between the UVJ and vagina, with the vagina harboring greater bacterial richness and distinct community structures compared with the UVJ. Administration of BAR did not significantly affect global microbial alpha diversity but was associated with selective enrichment of low-abundance taxa and functional pathways, particularly in the UVJ. These findings reveal previously unexplored host–microbe interactions in the avian reproductive tract and provide new insights into how antioxidant-mediated NRF2 activation may influence microbial functional capacity without destabilizing overall community structure.

Consistent with their distinct physiological roles, the UVJ and vagina harbored significantly different microbial communities. We observed that vagina exhibited greater bacterial richness compared with UVJ, while overall Shannon and Simpson indices were similar, suggesting comparable evenness across tissues. Beta diversity analyses confirmed strong separation of community structures



between UVJ and vagina, with more pronounced differences under unweighted Bray–Curtis distances, indicating that rare taxa contributed disproportionately to anatomical divergence. This aligns with prior reports in chickens and turkeys showing site-specific microbial communities along the reproductive tract (Lee et al., 2019; Shterzer et al., 2020; Kursu et al., 2022).

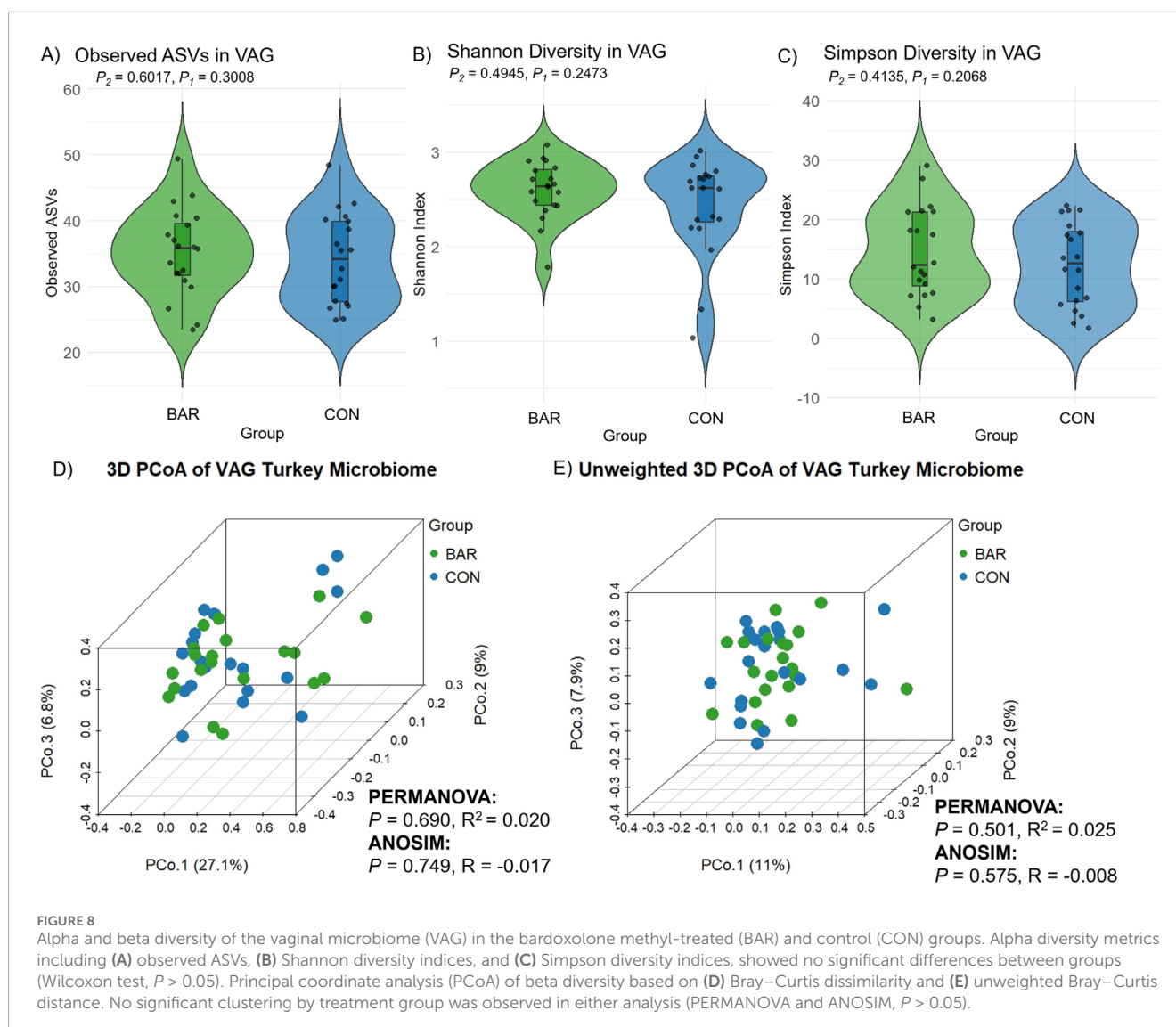
Previous studies in layer chickens reported *Firmicutes*, *Proteobacteria*, and *Cyanobacteria* as dominant phyla in the avian reproductive tract (Su et al., 2021b). Similarly, in broiler breeders of the Cobb breed at 37 weeks of age (Shterzer et al., 2020) and in 32-week-old laying SPF hens (Lee et al., 2019), *Firmicutes* was identified as the predominant phylum. Slightly different results



were observed in the turkey oviduct, where the dominant phyla were *Proteobacteria*, *Firmicutes*, *Actinobacteria*, and *Bacteroidetes* (Kursa et al., 2022). Comparing the bacterial composition of turkey oviducts with that of chickens, certain phyla in low abundance, such as *Planctomycetes*, were detected in both species (Lee et al., 2019). In addition, *Firmicutes* and *Proteobacteria* were identified as the dominant phyla in the turkey respiratory tract (Kursa et al., 2021). Other low-abundance phyla, including *Fusobacteria*, *Cyanobacteria*, *Acidobacteria*, *Chloroflexi*, and *Deinococcus-Thermus*, have also been reported in the avian respiratory tract (Shabbir et al., 2015). Notably, these phyla *Firmicutes*, *Proteobacteria*, *Thermoproteota*, and *Actinobacteriota* were the dominant microbiota in turkey lower reproductive tract in this study.

Taxonomic profiling highlighted tissue-specific colonization patterns. Aamples from the UVJ were enriched in *Staphylococcus* and *Escherichia* ASVs, whereas VAG samples contained higher abundances of *Romboutsia_B.ilealis*, *Ligilactobacillus*, and *Limosilactobacillus*. The predominance of lactobacilli in the vagina is consistent with their well-documented role in producing lactic

acid and antimicrobial compounds that maintain a protective, low-pH environment, thereby restricting colonization by opportunistic pathogens (Boskey et al., 2001; Ravel et al., 2011; Su et al., 2021a). These taxonomic differences are in line with previous reports describing site-specific microbial communities along the avian reproductive tract, with the UVJ acting as a selective niche for sperm storage and protection (Holt and Fazeli, 2016; Shterzer et al., 2024). Comparable patterns of anatomical specialization have also been observed in broiler breeders, where distinct bacterial assemblages were detected between the magnum and infundibulum, highlighting the influence of localized microenvironments on shaping microbial composition (Wen et al., 2021; Shterzer et al., 2024). Beyond the dominant taxa in the turkey lower reproductive tract, several low-abundance genera and species exhibited tissue-specific enrichment, suggesting potential niche-specific functions. In the VAG, these included *Anaerococcus*, *Clostridium*, *Ruminococcaceae*, *Preptostreptococcaceae_256921* (*Firmicutes_A*), *Limosilactobacillus*, *ingluviei*, *Enterococcus* (*Firmicutes_D*), and *UBA6249* (*Omnitrophota*). In the UVJ, enrichment was observed



for *Limosilactobacillus fermentum* (Firmicutes_D), *UTCFX2.sp002050125* (Chloroflexota), and *UBA6902* (Nitrospirata). Although many of these taxa have not been previously reported in avian reproductive tissues, studies in other species indicate potential roles in mucosal metabolism, colonization resistance, and biofilm formation. For example, *Anaerococcus* (Firmicutes_A) has been reported in the vaginal microbiota of sows, where its abundance was associated with reproductive tract conditions such as pelvic organ prolapse during late gestation (Kiefer et al., 2021). While the host species differ, the repeated detection of *Anaerococcus* in mammalian and avian reproductive tracts suggests a conserved potential function in maintaining mucosal homeostasis or modulating local microbial communities. *Clostridium* and *Ruminococcaceae* (Firmicutes_A) are known SCFA producers in humans and bovines, which can lower vaginal pH and inhibit pathogen growth (Fusco et al., 2023; Gupta et al., 2024). *Limosilactobacillus fermentum* (Firmicutes_D) contributes to mucosal protection and pathogen inhibition in mice and humans (Rodríguez-Sojo et al., 2021; Kang et al., 2018), while *Enterococcus* (Firmicutes_D) have been

associated with nutrient metabolism and environmental adaptation in diverse hosts (Gupta et al., 2024).

Bardoxolone methyl administration did not significantly alter overall alpha diversity in either UVJ or vagina, consistent with a lack of broad community disruption. However, in the UVJ, unweighted beta diversity analyses revealed modest but significant separation between BAR and control groups, suggesting selective shifts in low-abundance taxa. Indeed, indicator species analysis identified increased *Corynebacterium* in bardoxolone methyl-treated birds, while *Pseudomonadaceae* and *Burkholderia* were more abundant in the controls. These findings are noteworthy because certain members of *Pseudomonas* and *Burkholderia* are known opportunists in avian reproductive systems and egg contents (Bassols et al., 2016; Eraky et al., 2020; Abd El-Ghany, 2021; Echols and Speer, 2022; Shterzer et al., 2024). Thus, bardoxolone methyl may promote a microbial environment less dominated by opportunistic taxa while favoring mucosa-adapted commensals such as *Corynebacterium*. In the vagina, neither alpha nor beta diversity metrics differed between groups, but BAR selectively

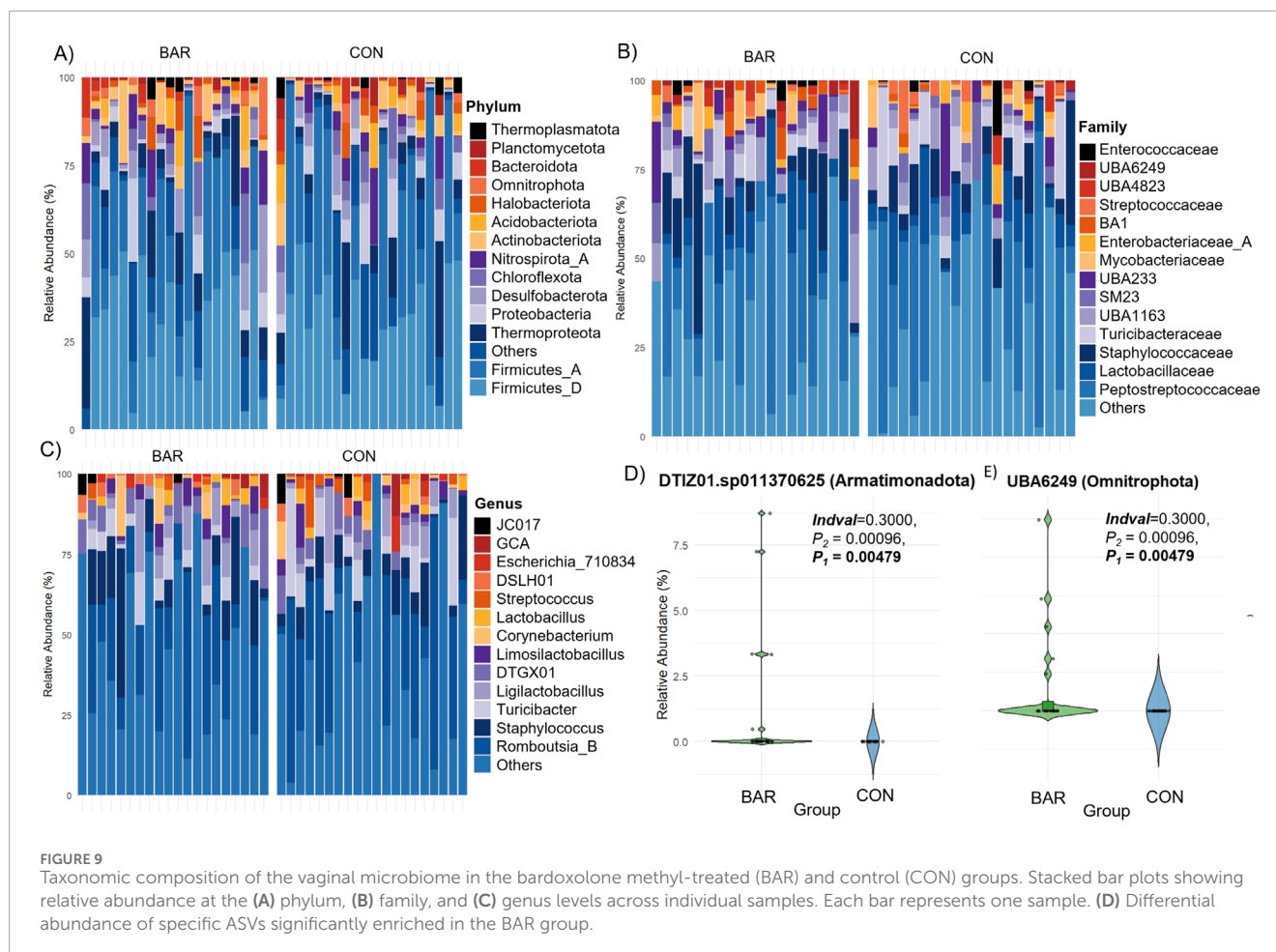


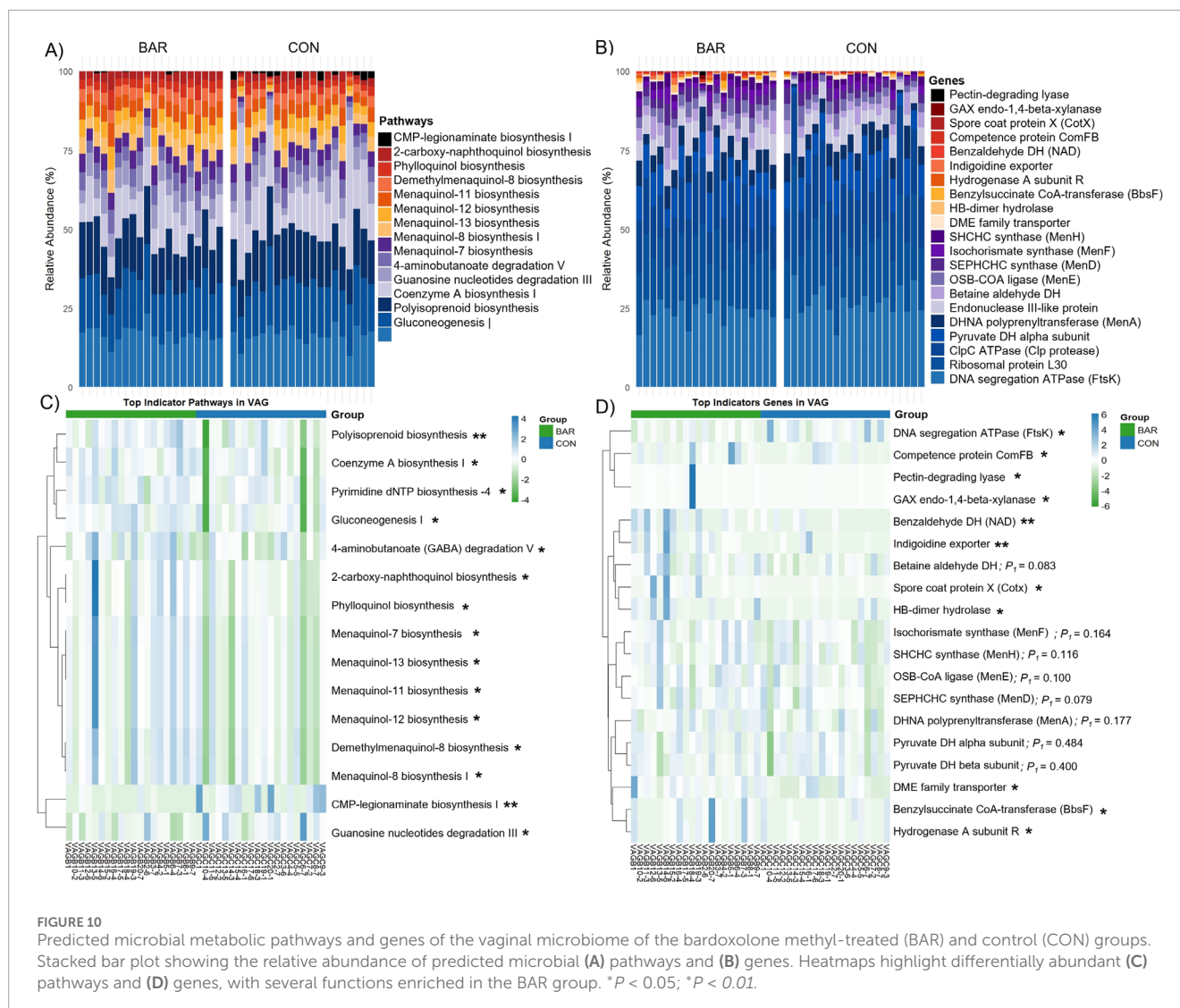
FIGURE 9
Taxonomic composition of the vaginal microbiome in the bardoxolone methyl-treated (BAR) and control (CON) groups. Stacked bar plots showing relative abundance at the (A) phylum, (B) family, and (C) genus levels across individual samples. Each bar represents one sample. (D) Differential abundance of specific ASVs significantly enriched in the BAR group.

enriched two rare taxa, *DTIZ01. sp011370625* (*Armatimonadota*) and *UBA6249* (*Omnitrophota*), paralleling the UVJ pattern of subtle selection on low-abundance members.

Predicted microbial pathways and genes revealed both shared core pathways and site-specific genes along the turkey lower reproductive tract. Core pathways, including the pentose phosphate pathway, adenosine *de novo* biosynthesis, and the Calvin-Benson-Bassham cycle, were abundant across both the UVJ and vagina, reflecting fundamental microbial energy metabolism and nucleotide biosynthesis (Stincone et al., 2015). Indicator pathways enriched in the vagina, such as dAMP synthesis II, Coenzyme A biosynthesis I, and glycolysis, suggest enhanced microbial proliferation, energy metabolism, and adaptation to anaerobic conditions, while UVJ-enriched genes, including sarcosine oxidase and choline dehydrogenase, support one-carbon metabolism, osmoprotection, and oxidative stress adaptation (Wu, 2009), potentially facilitating sperm storage. Bardoxolone methyl did not broadly alter overall pathway or gene diversity but selectively enriched functions related to energy metabolism, nucleotide biosynthesis, and regulatory systems for environmental sensing, consistent with microbiome functional resilience, in which microbes maintain core functions under fluctuating conditions without major shifts in community composition (Lozupone et al., 2012). In the UVJ, BAR enhanced carbohydrate and nucleotide metabolism along with regulatory pathways, including NAD

and isoprenoid biosynthesis, peptidoglycan biosynthesis, the ethylmalonyl-CoA pathway, consisting of Cell cycle transcriptional regulator A (CtrA), Cell cycle histidine kinase A (CckA), Histidine phosphotransferase ChpT, Cell cycle phosphodiesterase regulator CpdR, Chromosomal virulence sensor kinase ChvG, and Chromosomal virulence response regulator ChvI, indicating improved microbial adaptability to host signals, cell cycle control, and stress resilience (Hooper et al., 2012; Belkaid and Hand, 2014; Bhagat et al., 2025). In the vagina, BAR selectively enriched metabolic pathways such as menaquinone biosynthesis and polyisoprenoid biosynthesis, as well as genes including *pyruvate dehydrogenase*, *hydrogenase A*, and *betaine aldehyde dehydrogenase*, supporting energy production, redox balance, nucleotide turnover, protein homeostasis, and carbon substrate flexibility, thereby promoting microbial metabolic robustness, stress adaptation, and community maintenance (Meganathan and Kwon, 2009). Overall, these findings suggest that BAR reinforces microbiome functional resilience and mucosal stability without broadly altering composition, which may support genomic stability and protein homeostasis.

Our findings also highlight that avian reproductive tract microbiomes differ fundamentally from those in mammals. While human vaginal microbiota is typically dominated by *Lactobacillus* species (Ravel et al., 2011), our profiles confirm previous observations that the avian reproductive tract harbors a more diverse



community of *Firmicutes*, *Proteobacteria*, *Actinobacteriota*, and other taxa (Lee et al., 2019; Glendinning et al., 2020). This emphasizes the need for species-specific perspectives when interpreting microbial contributions to the function of the reproductive tract.

5 Conclusion

In conclusion, administration of bardoxolone methyl subtly but consistently modulated the turkey lower reproductive tract microbiome. This modulation primarily involved enrichment of rare taxa and functional pathways related to metabolism, redox balance, and environmental sensing, without disrupting overall microbial diversity. These findings are consistent with known NRF2 biology and suggest that antioxidant-mediated redox regulation can fine-tune mucosal microbial communities, particularly in the UVJ. While the present manuscript focuses on the effects of BAR on the microbial communities within the UVJ and vagina, we have also evaluated its influence on gene and protein expression in these tissues, as well as its impact on key reproductive traits. The outcomes

of these complementary analyses will be detailed in a separate manuscript. Future studies should integrate multi-omics approaches and reproductive performance metrics to validate the functional consequences of these microbial changes and to further elucidate host–microbe interactions relevant to fertility in poultry.

Data availability statement

All sequencing data supporting the findings of this study have been deposited in the NCBI Sequence Read Archive (SRA) under the BioProject accession number PRJNA1311734 and are available at <http://www.ncbi.nlm.nih.gov/bioproject/1311734>.

Ethics statement

The animal studies were approved by Institutional Animal Care and Use Committee. The studies were conducted in accordance with the local legislation and institutional requirements.

Written informed consent was obtained from the owners for the participation of their animals in this study.

Author contributions

JN: Data curation, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. AG: Data curation, Formal Analysis, Methodology, Resources, Software, Supervision, Visualization, Writing – review and editing. SR: Data curation, Formal Analysis, Software, Supervision, Writing – review and editing. PS: Investigation, Methodology, Supervision, Validation, Writing – review and editing. SK: Investigation, Methodology, Supervision, Validation, Writing – review and editing. MS: Investigation, Resources, Writing – review and editing. BW: Conceptualization, Investigation, Resources, Writing – review and editing. CN: Resources, Supervision, Writing – review and editing. KB: Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review and editing.

Funding

The author(s) declare that financial support was received for the research and/or publication of this article. This study was funded by the Minnesota Experiment Station Agricultural Rapid Response Funds project #RR269 and USDA-NIFA, Award No. 2025-67015-44285. JN received support from the National Research Council of Thailand (NRCT) through the Royal Golden Jubilee Ph.D. Program (Grant number: N41A661105).

Acknowledgements

We would like to thank Katie Fransen and her team at Select Genetics for their technical support in the preparation of compounds administered during the *in vivo* experiments. We also thank David Brown and Scott O'Grady for their guidance on the use of bardoxolone methyl *in vivo*. This manuscript was edited for clarity and style with the assistance of ChatGPT 5 (OpenAI, San Francisco,

CA), which was used solely to improve grammar and readability. The authors reviewed and approved all content.

Conflict of interest

Author MS, and BW was employed by Select Genetics.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declare that Generative AI was used in the creation of this manuscript. This manuscript was edited for clarity and style with the assistance of ChatGPT (OpenAI, San Francisco, CA), which was used solely to improve grammar and readability. The authors reviewed and approved all content.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2025.1703742/full#supplementary-material>

References

- Abd El-Ghany, W. A. (2021). *Pseudomonas aeruginosa* infection of avian origin: zoonosis and one health implications. *Vet. World* 14 (8), 2155–2159. doi:10.14202/vetworld.2021.2155-2159
- Andrews, S., Krueger, F., Segonds-Pichon, A., Biggins, L., Krueger, C., and Wingett, S. (2010). FastQC A quality control tool for high throughput sequence data. Available online at: <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/> (Accessed August 12, 2025).
- Bassols, J., Serino, M., Carreras-Badosa, G., Burcelin, R., Blasco-Baque, V., Lopez-Bermejo, A., et al. (2016). Gestational diabetes is associated with changes in placental microbiota and microbiome. *Pediatr. Res.* 80 (6), 777–784. doi:10.1038/pr.2016.155
- Belkaid, Y., and Hand, T. W. (2014). Role of the microbiota in immunity and inflammation. *Cell* 157 (1), 121–141. doi:10.1016/j.cell.2014.03.011
- Bhagat, N. R., Bharti, V. K., Shukla, G., Rishi, P., and Chaurasia, O. (2025). Gut bacteriome dynamics in high altitude-adapted chicken lines: a key to future poultry therapeutics. *Sci. Rep.* 15 (1), 11910. doi:10.1038/s41598-025-96178-1
- Biswas, S. K. (2016). Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? *Oxid. Med. Cell. Longev.* 2016 (1), 5698931. doi:10.1155/2016/5698931
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., et al. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* 37 (8), 852–857. doi:10.1038/s41587-019-0209-9
- Boskey, E., Cone, R., Whaley, K., and Moench, T. (2001). Origins of vaginal acidity: high D/L lactate ratio is consistent with bacteria being the primary source. *Hum. Reprod.* 16 (9), 1809–1813. doi:10.1093/humrep/16.9.1809
- Callahan, B., and Davis, N. (2025). Decontam: identify contaminants in marker-gene and metagenomics sequencing data. Available online at: https://bioconductor.org/packages/devel/bioc/vignettes/decontam/inst/doc/decontam_intro.html (Accessed August 12, 2025).

- Callahan, B. J., Mcmurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from illumina amplicon data. *Nat. Methods* 13 (7), 581–583. doi:10.1038/nmeth.3869
- Chen, S.-Y., Deng, F., Zhang, M., Jia, X., and Lai, S.-J. (2020). Characterization of vaginal microbiota associated with pregnancy outcomes of artificial insemination in dairy cows. *J. Microbiol. Biotechnol.* 30 (6), 804–810. doi:10.1016/j.jmb.2020.02010
- Chen, Y., Kosonsiriluk, S., Ehresmann, L. X., Reed, K. M., Noll, S. L., Wileman, B. W., et al. (2024). Artificial insemination and laying cycle influence adaptive immune cell numbers and localization in the reproductive tract of Turkey breeder hens. *Poult. Sci.* 103 (12), 104448. doi:10.1016/j.psj.2024.104448
- Christensen, V. (1981). Effect of insemination intervals on oviducal sperm storage in turkeys. *Poult. Sci.* 60 (9), 2150–2156. doi:10.3382/ps.0602150
- Deng, F., McClure, M., Rorie, R., Wang, X., Chai, J., Wei, X., et al. (2019). The vaginal and fecal microbiomes are related to pregnancy status in beef heifers. *J. Anim. Sci. Biotechnol.* 10 (1), 92. doi:10.1186/s40104-019-0401-2
- Douglas, G. M., Maffei, V. J., Zaneveld, J. R., Yurgel, S. N., Brown, J. R., Taylor, C. M., et al. (2020). PICRUSt2 for prediction of metagenome functions. *Nat. Biotechnol.* 38 (6), 685–688. doi:10.1038/s41587-020-0548-6
- Echols, S., and Speer, B. (2022). Avian reproductive tract diseases and surgical resolutions. *Clin. Theriogenology* 14 (1), 32–43. doi:10.58292/ct.v14.9297
- Eraky, R., Abd El-Ghany, W., and Soliman, K. (2020). Studies on *Pseudomonas aeruginosa* infection in hatcheries and chicken. *J. Hell. Vet. Med. Soc.* 71 (1), 1953–1962. doi:10.12681/jhvms.22937
- Food and Agriculture Organization of the United Nations (2023). *Crops and livestock products*. Available online at: <https://www.fao.org/faostat/en/#data/QCL> (Accessed October 14, 2025).
- Fusco, W., Lorenzo, M. B., Cintoni, M., Porcari, S., Rinninella, E., Kaitsas, F., et al. (2023). Short-chain fatty-acid-producing bacteria: key components of the human gut microbiota. *Nutrients* 15 (9), 2211. doi:10.3390/nu15092211
- García-Velasco, J. A., Menabrito, M., and Catalán, I. B. (2017). What fertility specialists should know about the vaginal microbiome: a review. *Reprod. Biomed. Online* 35 (1), 103–112. doi:10.1016/j.rbmo.2017.04.005
- Glendinning, L., Stewart, R. D., Pallen, M. J., Watson, K. A., and Watson, M. (2020). Assembly of hundreds of novel bacterial genomes from the chicken caecum. *Genome Biol.* 21 (1), 34. doi:10.1186/s13059-020-1947-1
- Gohl, D. M., Vangay, P., Garbe, J., Maclean, A., Hauge, A., Becker, A., et al. (2016). Systematic improvement of amplicon marker gene methods for increased accuracy in microbiome studies. *Nat. Biotechnol.* 34 (9), 942–949. doi:10.1038/nbt.3601
- Gupta, D., Sarkar, A., Pal, Y., Suthar, V., Chawade, A., and Kushwaha, S. K. (2024). Bovine reproductive tract and microbiome dynamics: current knowledge, challenges, and its potential to enhance fertility in dairy cows. *Front. Microbiomes* 3, 1473076. doi:10.3389/fmibi.2024.1473076
- Holt, W. V., and Fazeli, A. (2016). Sperm storage in the female reproductive tract. *Annu. Rev. Anim. Biosci.* 4 (1), 291–310. doi:10.1146/annurev-animal-021815-111350
- Hooper, L. V., Littman, D. R., and Macpherson, A. J. (2012). Interactions between the microbiota and the immune system. *science* 336 (6086), 1268–1273. doi:10.1126/science.1223490
- Kang, C.-H., Kim, Y., Han, S. H., Kim, J.-S., Paek, N.-S., and So, J.-S. (2018). *In vitro* probiotic properties of vaginal *Lactobacillus fermentum* MG901 and *Lactobacillus plantarum* MG989 against *Candida albicans*. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 228, 232–237. doi:10.1016/j.ejogrb.2018.07.005
- Khansari, N., Shakiba, Y., and Mahmoudi, M. (2009). Chronic inflammation and oxidative stress as a major cause of age-related diseases and cancer. *Recent Pat. Inflamm. Allergy Drug Discov.* 3 (1), 73–80. doi:10.2174/187221309787158371
- Kiefer, Z. E., Koester, L. R., Studer, J. M., Chipman, A. L., Mainquist-Whigham, C., Keating, A. F., et al. (2021). Vaginal microbiota differences associated with pelvic organ prolapse risk during late gestation in commercial sows. *Biol. Reprod.* 105 (6), 1545–1561. doi:10.1093/biolre/iaob178
- Kim, S. O., Sheikh, H. I., Ha, S. D., Martins, A., and Reid, G. (2006). G-CSF-mediated inhibition of JNK is a key mechanism for *Lactobacillus rhamnosus*-induced suppression of TNF production in macrophages. *Cell. Microbiol.* 8 (12), 1958–1971. doi:10.1111/j.1462-5822.2006.00763.x
- Kolde, R. (2025). *Pheatmap: pretty Heatmaps. R package version 1.0.13*. Available online at: <https://cran.r-project.org/web/packages/pheatmap/pheatmap.pdf> (Accessed August 12, 2025).
- Kosonsiriluk, S., Reed, K. M., Noll, S. L., Wileman, B. W., Studniski, M. M., and Boukherroub, K. S. (2023). Prolonged repeated inseminations trigger a local immune response and accelerate aging of the uterovaginal junction in Turkey hens. *Front. Physiol.* 14, 1275922. doi:10.3389/fphys.2023.1275922
- Kursa, O., Tomczyk, G., Sawicka-Durkalec, A., Giza, A., and Słomiany-Szwarc, M. (2021). Bacterial communities of the upper respiratory tract of turkeys. *Sci. Rep.* 11 (1), 2544. doi:10.1038/s41598-021-81984-0
- Kursa, O., Tomczyk, G., Sawicka-Durkalec, A., and Adamska, K. (2022). Bacterial communities of the oviduct of turkeys. *Sci. Rep.* 12 (1), 14884. doi:10.1038/s41598-022-19268-4
- Langille, M. G., Zaneveld, J., Caporaso, J. G., McDonald, D., Knights, D., Reyes, J. A., et al. (2013). Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* 31 (9), 814–821. doi:10.1038/nbt.2676
- Lee, S., La, T., Lee, H., Choi, I., Song, C., Park, S., et al. (2019). Characterization of microbial communities in the chicken oviduct and the origin of chicken embryo gut microbiota. *Sci. Rep.* 9, 6838–11. doi:10.1038/s41598-019-43280-w
- Lozupone, C. A., Stombaugh, J. I., Gordon, J. I., Jansson, J. K., and Knight, R. (2012). Diversity, stability and resilience of the human gut microbiota. *Nature* 489 (7415), 220–230. doi:10.1038/nature11550
- M Jones, R., W Mercante, J., and S Neish, A. (2012). Reactive oxygen production induced by the gut microbiota: pharmacotherapeutic implications. *Curr. Med. Chem.* 19 (10), 1519–1529. doi:10.2174/092986712799828283
- Marciano, F., and Vajro, P. (2017). Oxidative stress and gut microbiota** conflict of interest: none. *Gastrointest. tissue*, 113–123. doi:10.1016/B978-0-12-805377-5.00008-4
- Martin, M. (2011). Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. J.* 17 (1), 10–12. doi:10.14806/ej.17.1.200
- Mcmurdie, P. J., and Holmes, S. (2013). Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS ONE* 8 (4), e61217. doi:10.1371/journal.pone.0061217
- Meganathan, R., and Kwon, O. (2009). Biosynthesis of menaquinone (vitamin K2) and ubiquinone (coenzyme Q). *EcoSal. Plus* 3 (2). doi:10.1128/ecosalplus.3.6.3.3
- Nangaku, M., Kanda, H., Takama, H., Ichikawa, T., Hase, H., and Akizawa, T. (2020). Randomized clinical trial on the effect of bardoxolone methyl on GFR in diabetic kidney disease patients (TSUBAKI study). *Kidney Int. Rep.* 5 (6), 879–890. doi:10.1016/j.ekir.2020.03.030
- O'Connell, R. M., Saha, S. K., and Cheng, G. (2005). Combating bacterial pathogens through host defense gene programs. *Curr. Immunol. Rev.* 1 (1), 43–54. doi:10.2174/1573395052952950
- Oksanen, J., Blanchet, F. G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., et al. (2025). *Vegan: Community ecology package version 2*. doi:10.32614/CRAN.package.vegan
- Paradis, E., Blomberg, S., Bolker, B., Brown, J., Claude, J., Cuong, H. S., et al. (2019). *Package 'ape'*. Available online at: <https://cran.stat.unipd.it/web/packages/ape/ape.pdf> (Accessed August 12, 2025).
- Pei, X., Zhang, X.-J., and Chen, H.-M. (2019). Bardoxolone treatment alleviates lipopolysaccharide (LPS)-induced acute lung injury through suppressing inflammation and oxidative stress regulated by Nrf2 signaling. *Biochem. Biophys. Res. Commun.* 516 (1), 270–277. doi:10.1016/j.bbrc.2019.06.006
- Ravel, J., Gajer, P., Abdo, Z., Schneider, G. M., Koenig, S. S., Mcculle, S. L., et al. (2011). Vaginal microbiome of reproductive-age women. *Proc. Natl. Acad. Sci.* 108 (Suppl. ment_1), 4680–4687. doi:10.1073/pnas.1002611107
- Roberts, D. W. (2019). *Labdsv: ordination and multivariate analysis for ecology*. Available online at: <https://CRAN.R-project.org/package=labdsv> (Accessed August 12, 2025).
- Robledinos-Antón, N., Fernández-Ginés, R., Manda, G., and Cuadrado, A. (2019). Activators and inhibitors of NRF2: a review of their potential for clinical development. *Oxid. Med. Cell. Longev.* 2019 (1), 9372182. doi:10.1155/2019/9372182
- Rodríguez-Sojo, M. J., Ruiz-Malagón, A. J., Rodríguez-Cabezas, M. E., Gálvez, J., and Rodríguez-Nogales, A. (2021). *Limosilactobacillus fermentum* CECT5716: mechanisms and therapeutic insights. *Nutrients* 13 (3), 1016. doi:10.3390/nu13031016
- Ruiz, S., Pergola, P. E., Zager, R. A., and Vaziri, N. D. (2013). Targeting the transcription factor Nrf2 to ameliorate oxidative stress and inflammation in chronic kidney disease. *Kidney Int.* 83 (6), 1029–1041. doi:10.1038/ki.2012.439
- Sanglard, L., Schmitz-Esser, S., Gray, K., Linhares, D., Yeoman, C., Dekkers, J., et al. (2020). Vaginal microbiota diverges in sows with low and high reproductive performance after porcine reproductive and respiratory syndrome vaccination. *Sci. Rep.* 10 (1), 3046. doi:10.1038/s41598-020-59955-8
- Sasanami, T., Matsuzaki, M., Mizushima, S., and Hiyama, G. (2013). Sperm storage in the female reproductive tract in birds. *J. Reprod. Dev.* 59 (4), 334–338. doi:10.1262/jrd.2013-038
- Shabbir, M. Z., Malys, T., Ivanov, Y. V., Park, J., Shabbir, M. a.B., Rabbani, M., et al. (2015). Microbial communities present in the lower respiratory tract of clinically healthy birds in Pakistan. *Poult. Sci.* 94 (4), 612–620. doi:10.3382/ps/pev010
- Shterzer, N., Rothschild, N., Sbehat, Y., Stern, E., Nazarov, A., and Mills, E. (2020). Large overlap between the intestinal and reproductive tract microbiomes of chickens. *Front. Microbiol.* 11, 1508. doi:10.3389/fmicb.2020.01508
- Shterzer, N., Sbehat, Y., Poudel, B., Rothschild, N., Oloko, O., Druyan, S., et al. (2024). Comparative analysis of reproductive tract microbiomes in modern and slower-growing broiler breeder lines. *Front. Vet. Sci.* 11, 1386410. doi:10.3389/fvets.2024.1386410

- Stincone, A., Prigione, A., Cramer, T., Wamelink, M. M., Campbell, K., Cheung, E., et al. (2015). The return of metabolism: biochemistry and physiology of the pentose phosphate pathway. *Biol. Rev.* 90 (3), 927–963. doi:10.1111/brv.12140
- Su, Y., Ge, Y., Xu, Z., Zhang, D., and Li, D. (2021a). The digestive and reproductive tract microbiotas and their association with body weight in laying hens. *Poult. Sci.* 100 (11), 101422. doi:10.1016/j.psj.2021.101422
- Su, Y., Tian, S., Li, D., Zhu, W., Wang, T., Mishra, S. K., et al. (2021b). Association of female reproductive tract microbiota with egg production in layer chickens. *Gigascience* 10 (9), giab067–16. doi:10.1093/gigascience/giab067
- Wen, C., Li, Q., Lan, F., Li, X., Li, G., Yan, Y., et al. (2021). Microbiota continuum along the chicken oviduct and its association with host genetics and egg formation. *Poult. Sci.* 100 (7), 101104. doi:10.1016/j.psj.2021.101104
- Wickham, H., Chang, W., Henry, L., Pedersen, L. T., Takahashi, K., Wilke, C., et al. (2025). *Package 'ggplot2'*. Available online at: <https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf> (Accessed August 12, 2025).
- Witkin, S. S., Linhares, I. M., Giraldo, P., and Ledger, W. J. (2007). An altered immunity hypothesis for the development of symptomatic bacterial vaginosis. *Clin. Infect. Dis.* 44 (4), 554–557. doi:10.1086/511045
- Wu, G. (2009). Amino acids: metabolism, functions, and nutrition. *Amino Acids* 37 (1), 1–17. doi:10.1007/s00726-009-0269-0