Exploring Probiotics and Upper Respiratory Tract Microbiome Interactions for Healthy Chickens without Antibiotics

Madhusudan Timilsina

Department of Veterinary Medicine,

University of Maryland, College Park

Virginia-Maryland College of Veterinary Medicine

Outline

Background

- Probiotics and Poultry Health
- Chicken URT and Microbiome
- Infectious Coryza: URT Infection model

Objectives

Impact of infection (*Avibacterium paragalinarum)* on the Upper Respiratory Tract Microbiome of Chickens

Methods

- Experimental Design
- Key Measures

Results

- Impact of infection on URT microbiome
- Strain-specific Impacts
- Temporal pattern in microbiome change

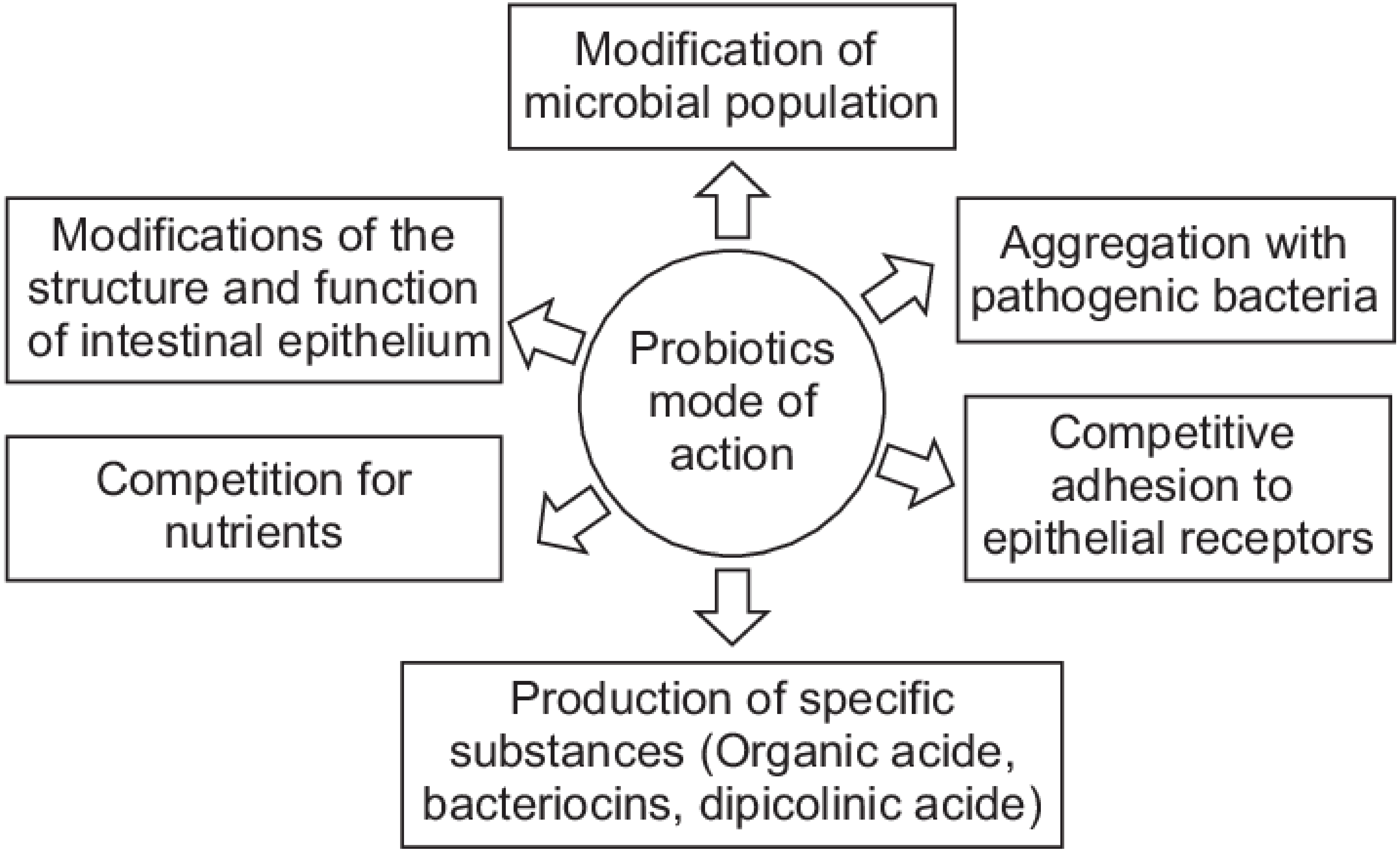
Conclusion and Next Steps

- URT microbiome changes following infection.
- Probiotics can be used to mitigate AP infections

What are probiotics?

- Live, non-pathogenic microbes that confer health benefits when administered in appropriate amounts.
- Include various species of *Lactobacillus*, *Bifidobacterium*, *Bacillus*, and *Enterococcus, etc.*(Stavropoulou and Bezirtzoglou, 2020).
- Primarily used for mitigating gut diseases in poultry, with growing research on respiratory health benefits.

Probiotics: How they work?



Probiotics mode of action

Modification of microbial population

Aggregation with pathogenic bacteria

Competitive adhesion to epithelial receptors

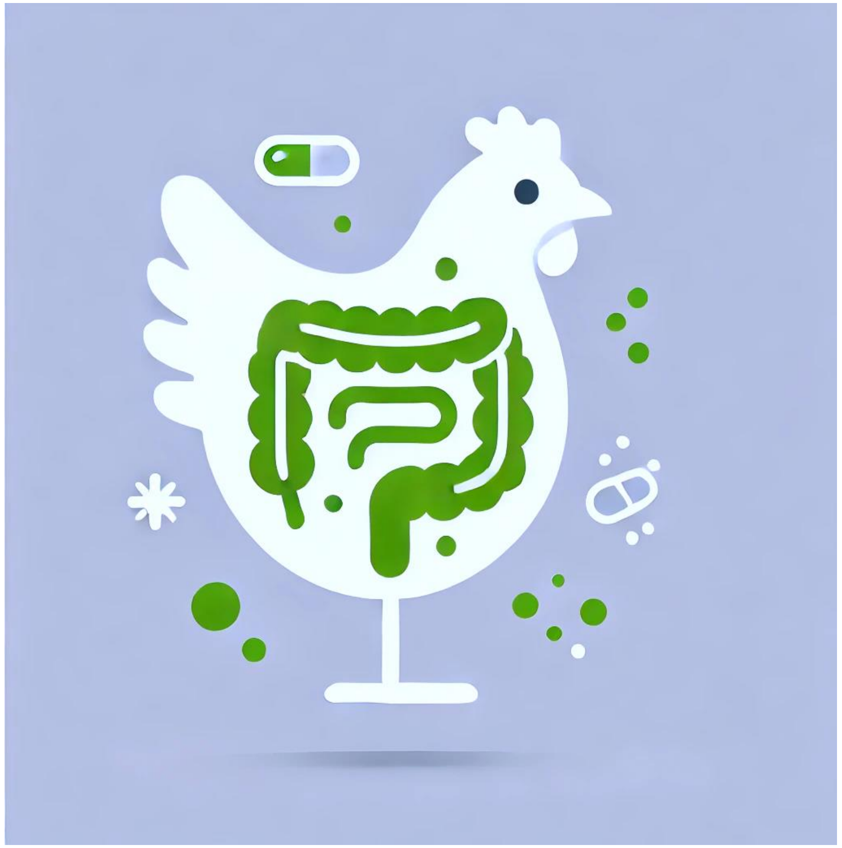
Production of specific substances (Organic acide, bacteriocins, dipicolinic acide)

Competition for nutrients

Modifications of the structure and function of intestinal epithelium

Source: Tiwari et al., 2012

Probiotics: Effects on Poultry Health



Source: NCH Life Sciences

- Lower the incidence of various enteric diseases.
- Enhance immune response
- Improves nutrient absorption, stress resilience, and overall productivity (Ayana & Kamutambuko, 2024).
- However, studies on using probiotics to modulate the Upper Respiratory Tract microbiome for disease mitigation are limited.

Chicken Upper Respiratory Tract (URT) & Microbiome

- Comprises the external nares, nasal cavity, oropharynx, larynx and trachea.
- Harbors rich microbial community (Oladokon and Sharif, 2024).
- Altered microbiome in the URT can lead to secondary infections or exacerbation of primary diseases in poultry (Kursa et al., 2022) .

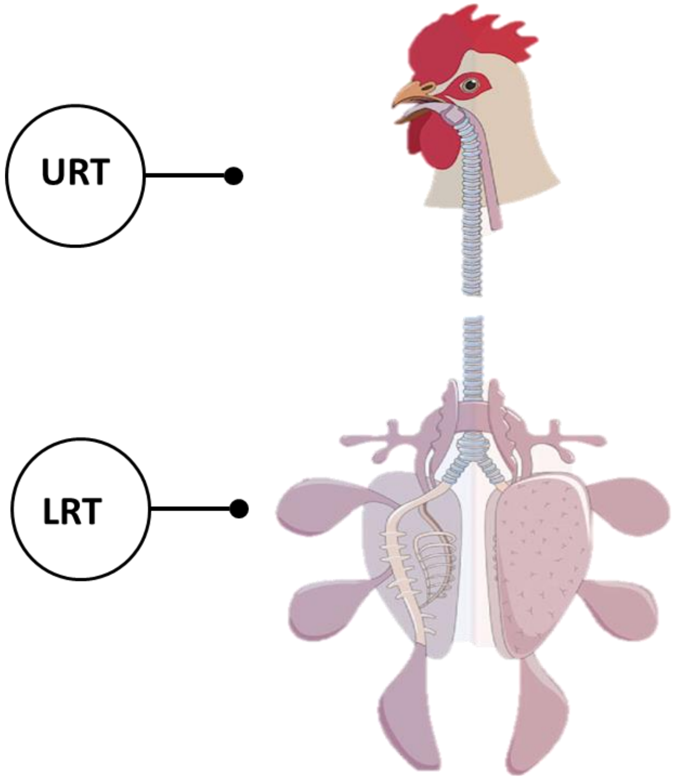


Figure: *Anatomy of the respiratory tract of chickens [source: Mach et al., 2021]*

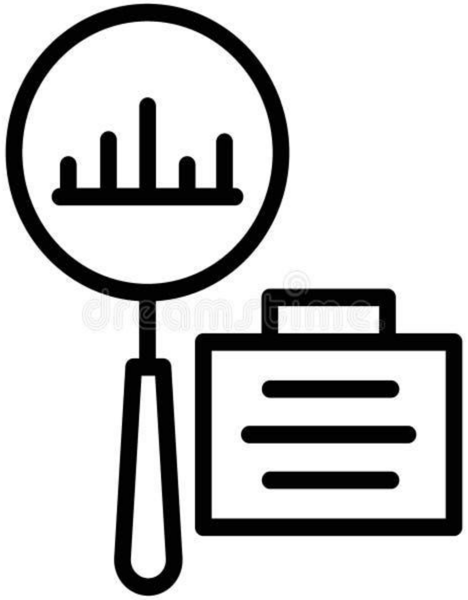
Infectious Coryza (IC), an URT infection model

- Etiology: *Avibacterium paragallinarum* (AP)
- Transmitted through aerosols containing AP and primarily affects the URT.
- Symptoms: Swollen head, nasal discharge, lachrymation, reduced feed and water consumption

Figure 2. *Clinical signs of Infectious Coryza in Chickens [source: Huo et al., 2023]*

Knowledge Gaps

- Limited studies on the effect of probiotics/infection on URT microbiome in chickens.
- Impact of AP infection on URT microbiome is unknown.
- Unclear if different strains of AP influence the microbiome differently



Objectives

Impact of infection (Avibacterium paragalinarum) on the Upper Respiratory Tract Microbiome of Chickens

- Evaluate the impact of AP infection on URT microbiome
- Evaluate the impact of different strains of AP on URT microbiome
- Determine temporal changes in URT microbiome during infection

Methods: Experimental Design

- Five groups of 28-day-old SPF chickens
  - Four groups infected with different *Avibacterium paragallinarum* (AP) strains.
  - One uninfected control group

- Intra-nasal instillation with 10^7 CFU of AP for challenge infection.

- Choanal swabs collected on days 2, 4, 7, and 9 post-challenge

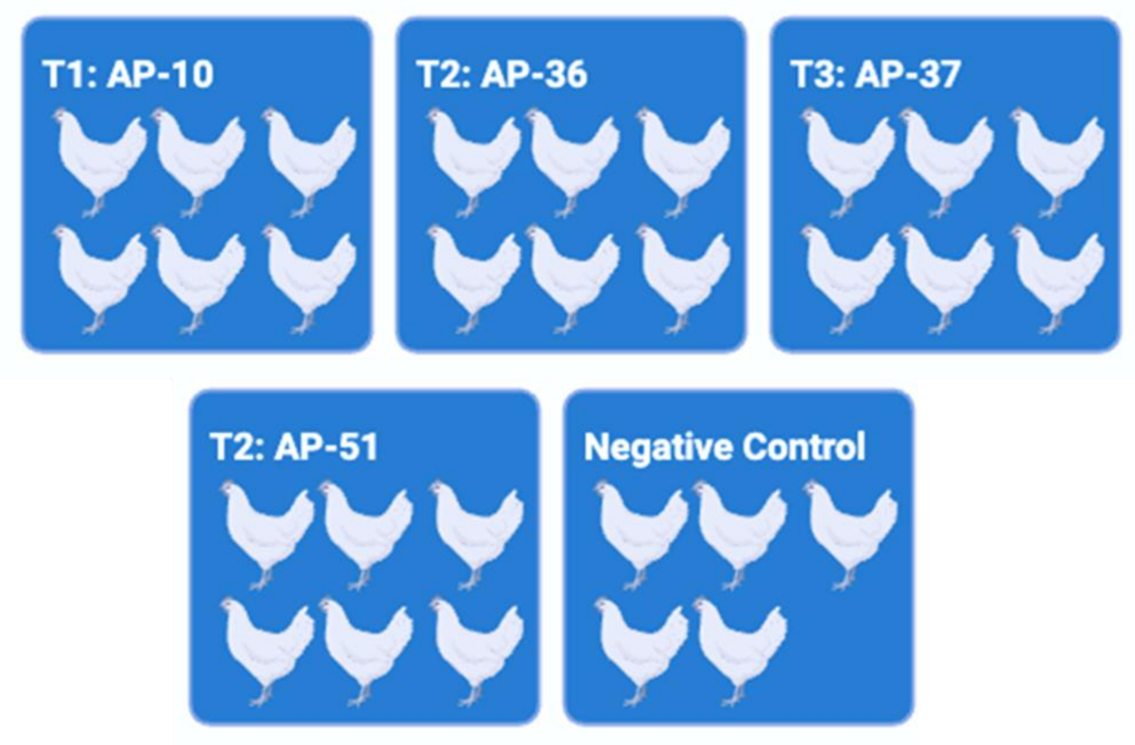
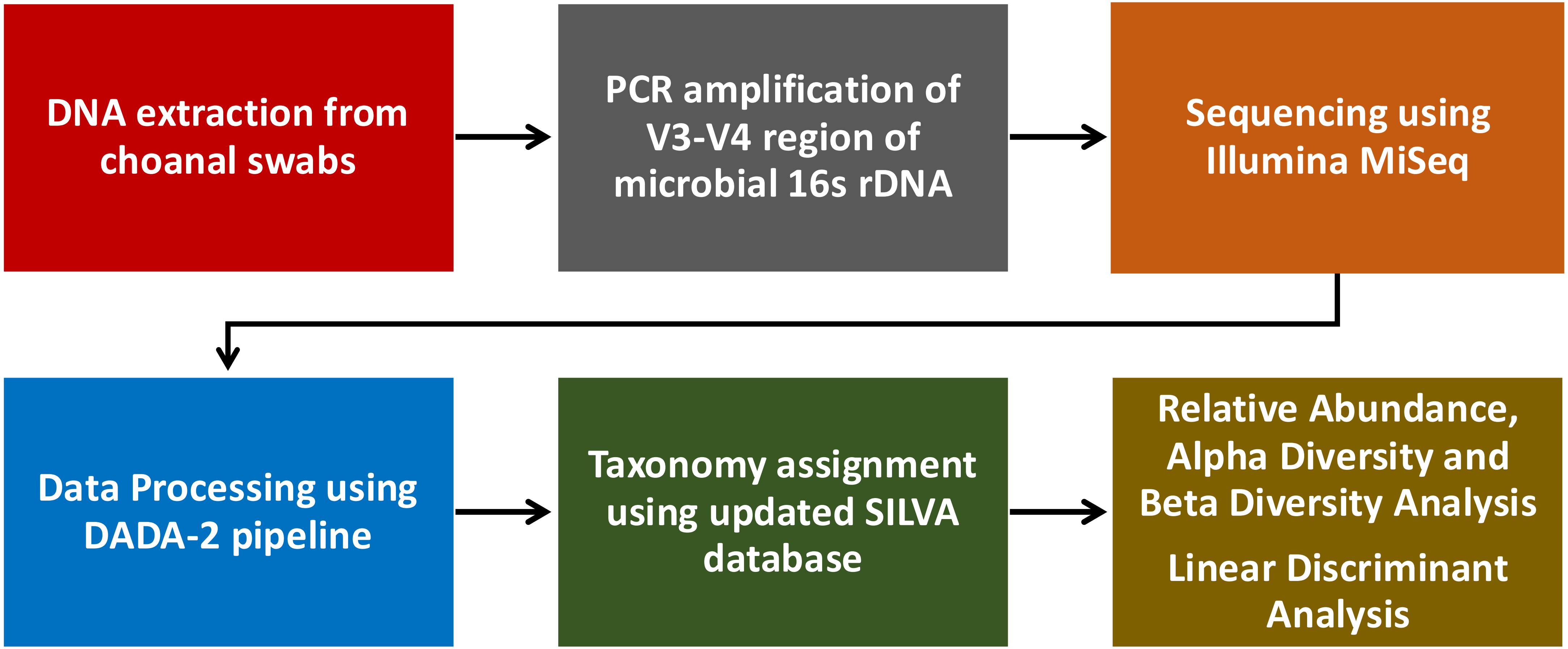


Figure : *Experimental groups designed for the challenge trial in 28-days old specific pathogen free (SPF) chickens*

Methods: Experimental Design



DNA extraction from choanal swabs

PCR amplification of V3-V4 region of microbial 16s rDNA

Sequencing using Illumina MiSeq

Data Processing using DADA-2 pipeline

Taxonomy assignment using updated SILVA database

Relative Abundance, Alpha Diversity and Beta Diversity Analysis

Linear Discriminant Analysis

Microbial Composition Measures

Relative Abundance

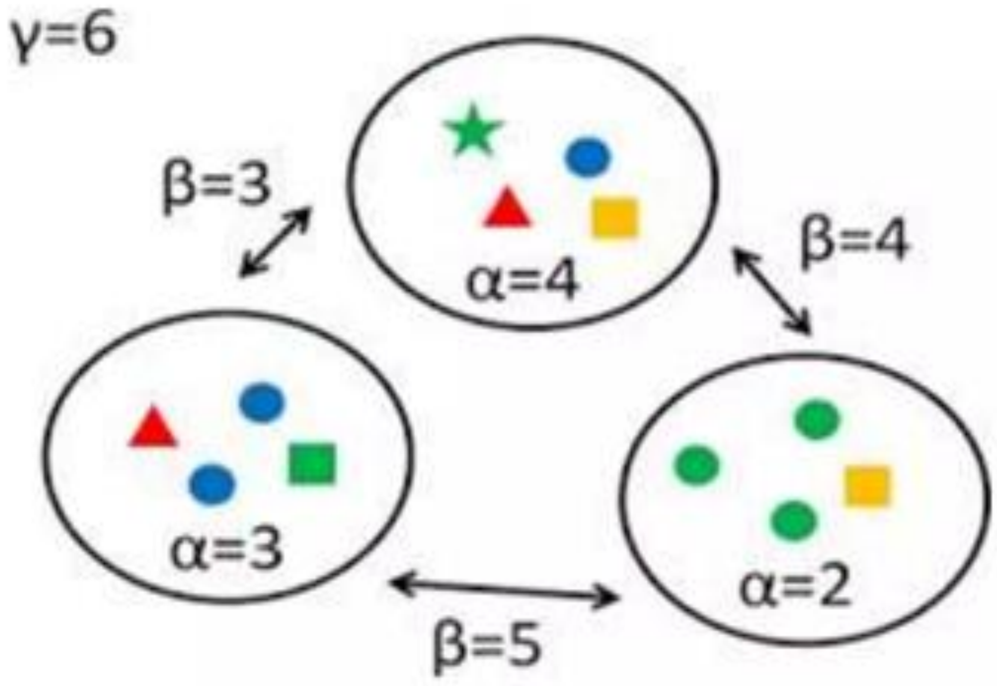
- Measures the proportion of each bacterial species within a community.

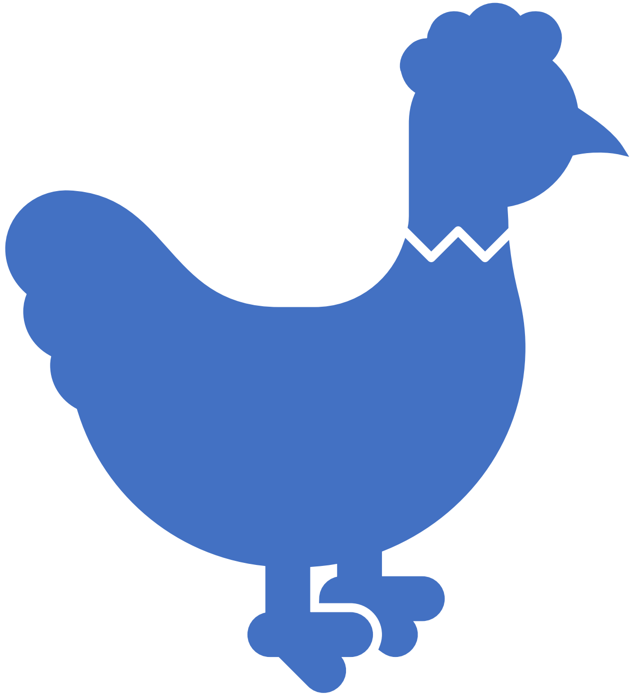
Alpha Diversity

- Measures how many different types of bacteria are present in a single chicken’s or a group respiratory tract.

Beta Diversity

- Measures how similar or different the bacterial communities are between different chickens or groups of chickens.





Results

1. Impact of AP Infection in the URT Microbiome of Chickens

Firmicutes, followed by Proteobacteria, Actinobacteria and Bacteroidota were the dominant phyla in the chicken URT

Figure: *Relative abundance of different phyla in the URT microbiome*

AP was highly abundant in 2 groups(AP-10 and AP-36) while negligible in other 2 groups

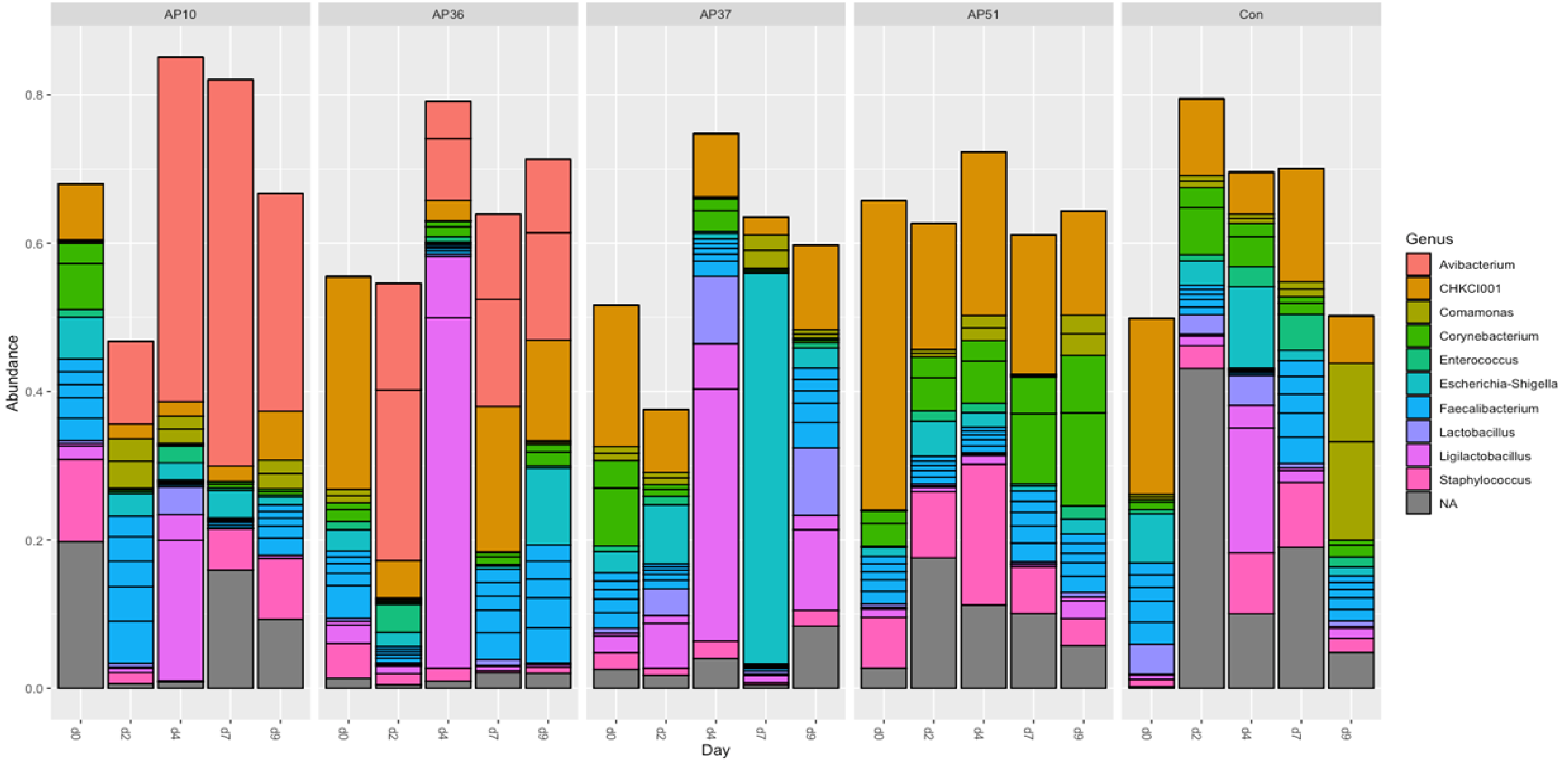


***Figure :** Relative abundance of the top-20 genera in the URT microbiome*

AP and two genera of *Lactobacillaceae* were highly enriched in infected chickens.

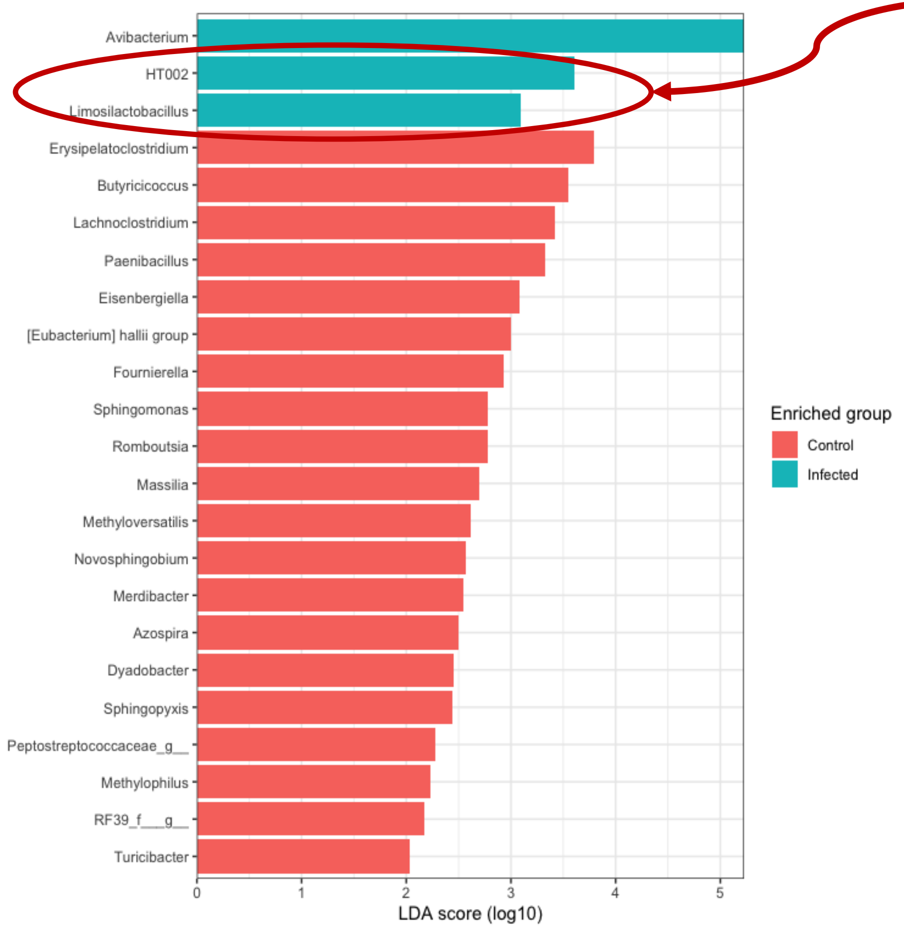


Figure: *LEfSE plot shows the genera differentially abundant in the infected and non-infected groups with LDA score >2*

- ○ HT002 and *Limosilactobacillus*: Genera within *Lactobacillaceae*, associated with the oral and gut microbiomes, respectively.
- ○ Although the mechanism is not clear, *Lactobacillaceae* was also found to be enriched during acute H9N2 avian influenza infection (Davis et al., 2023).
- ○ Further study needed. (if cause or result of infection)

dant

Reduced microbial diversity and altered community composition due to AP infection in chicken URT

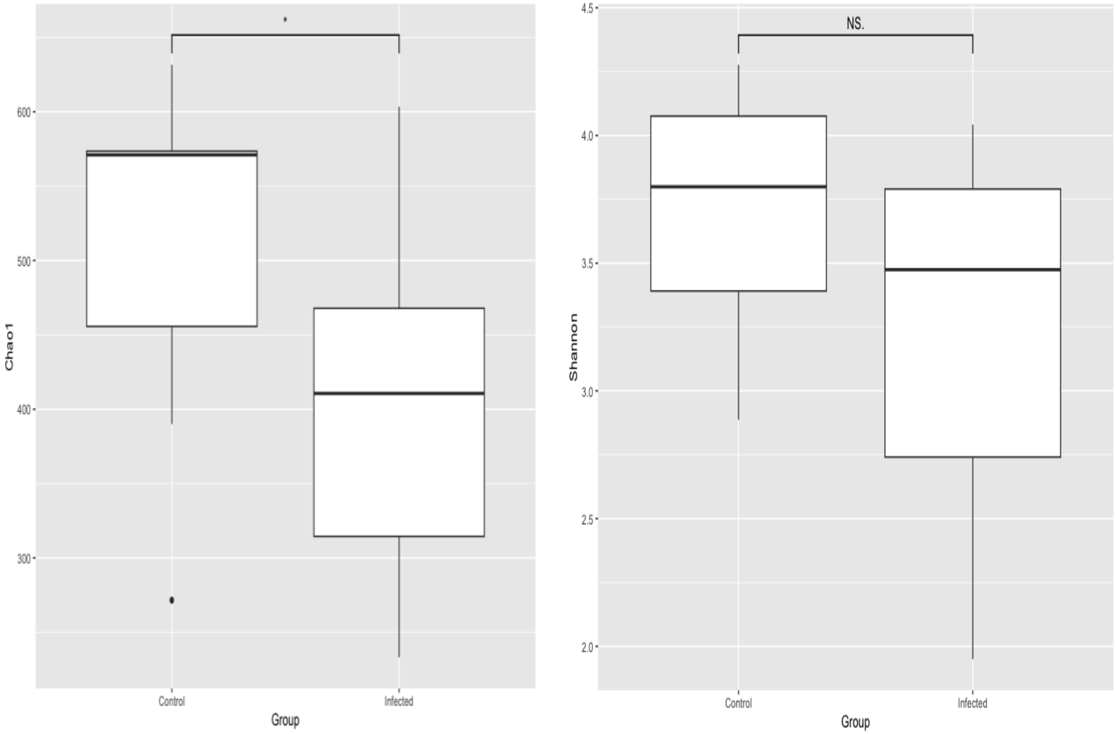


***Figure :** α-diversity of the microbiota in the Infected and Uninfected chickens by Chao1 and Shannon Index was significantly different by chao1 index (p= p=0.03145)*

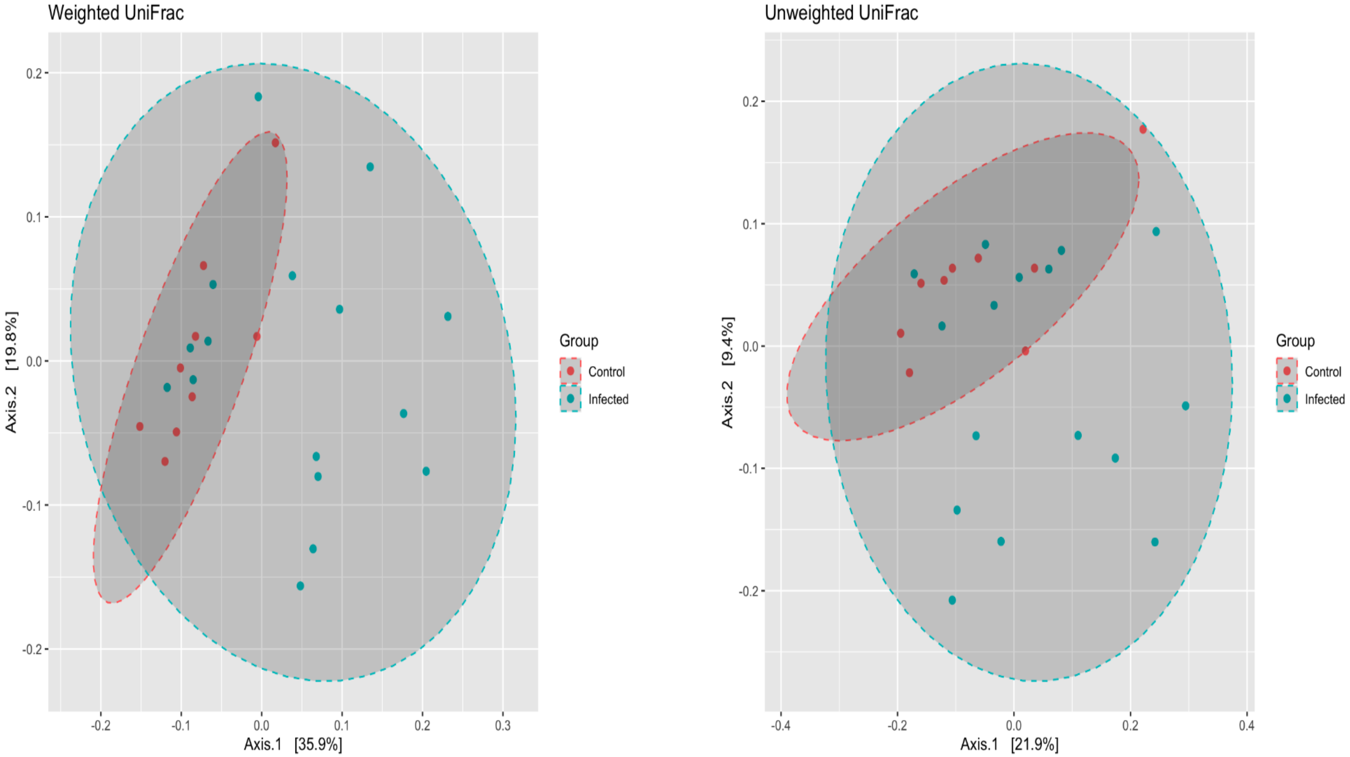
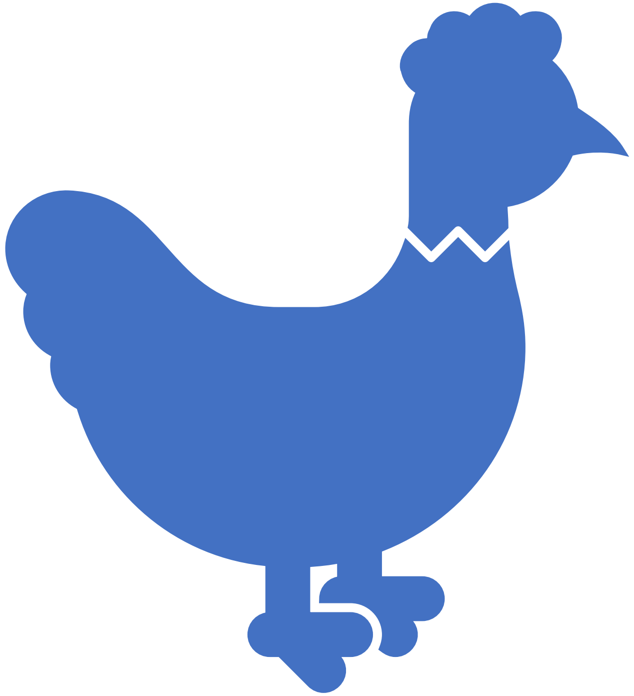


***Figure :** β-diversity between the infected and uninfected control groups by Weighted and Unweighted UniFrac- distances was significantly different (p= 0.014 and p=0.028 respectively)*



Results

2. Impact of Different Strains in the URT Microbiome of Chickens

Infection by different AP strains resulted in distinct microbiome profiles.

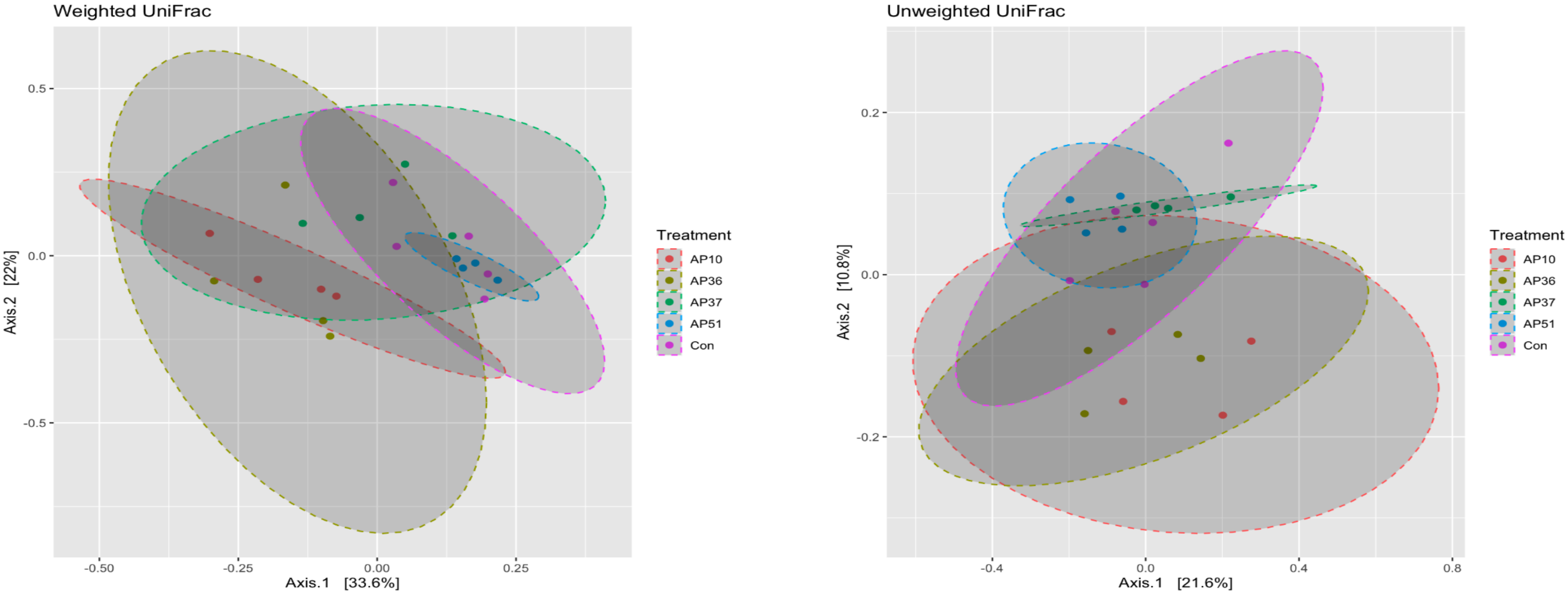
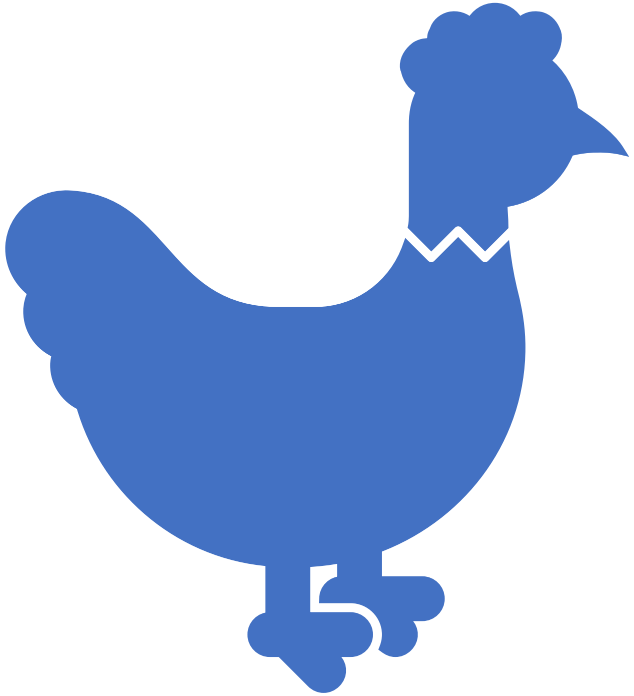


Figure : *β-diversity of the URT microbiome after infection by different strains of AP by Weighted and Unweighted UniFrac- distances was significantly different (p=0.001)*



Results

3. Temporal Changes in the URT Microbiome during the course of Infection

AP infection reduced alpha diversity during early days followed by gradual return to normal level

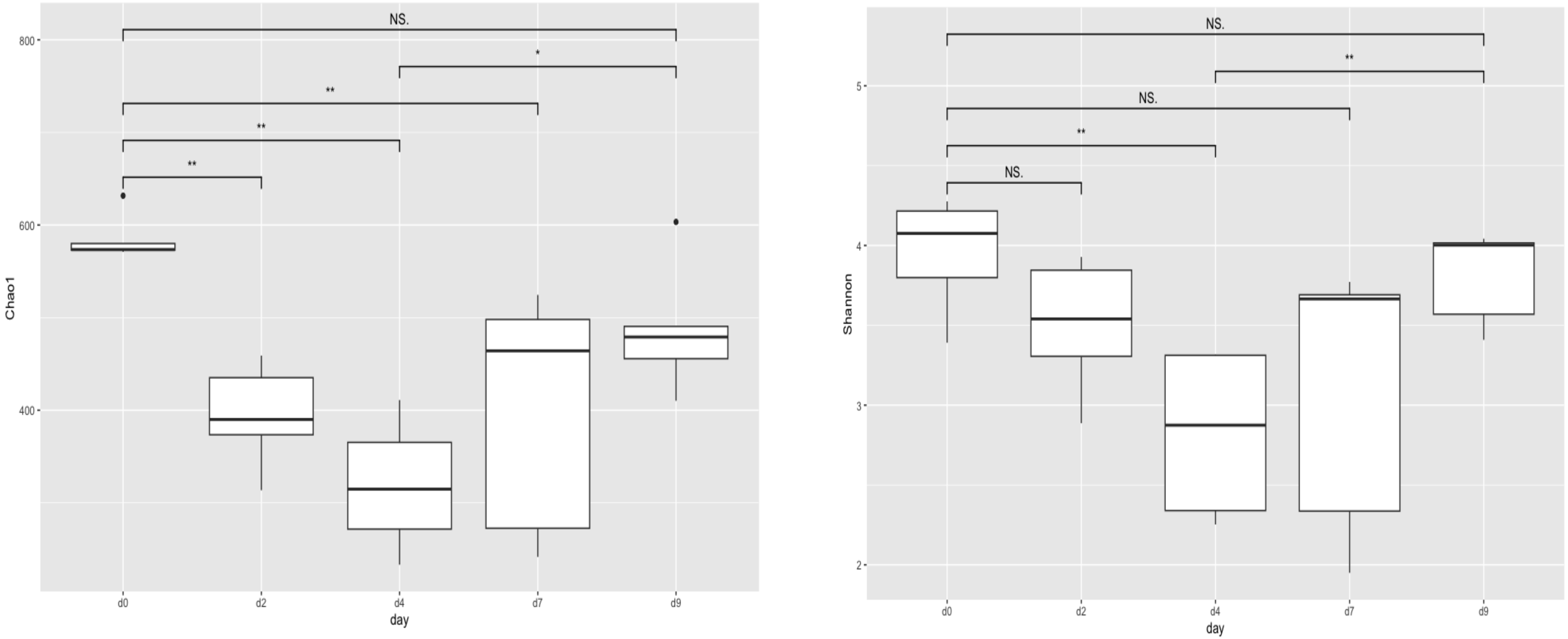


Figure : *α-diversity of the URT microbiome during IC infection by Chao1 and Shannon Index was significantly different between the different days*

Conclusion and Next Steps

- The URT of the chicken has a highly diverse and complex microbiome
- AP infection reduces richness and alters community composition.
- The alteration of URT microbiome found to be strain-specific and has temporal pattern.
- Two genera of *Lactobacillaceae* enriched in the infected chickens and further work needed to understand their role.

Next Steps

- Evaluate Naturally Attenuated AP Strains as Live Vaccine Candidates
- Evaluate the use of 3 identified probiotic candidates to mitigate AP infection in poultry

Long Term Impact

1. Probiotics could be a promising antibiotic alternative to mitigate for URT infections
2. Reduction in antibiotic use reduction of AMR Enhanced poultry health and productivity

Acknowledgment

Current & previous lab members

- Dr Mostafa Ghanem (PI)
- Dhiraj Chundru
- Alysaa Harris

Collaborators

- Dr. Shankar Mondal
- Dr. Yan Zhang
- Dr. Daniel Bautista, Zoetis

Funding

UNIVERSITY OF MARYLAND

CENTER OF EXCELLENCE IN MICROBIOME SCIENCES

NORTHEAST SARE Sustainable Agriculture Research & Education

Thank You

References

- Ayana, G. U., & Kamutambuko, R. (2024). Probiotics in disease management for sustainable poultry production. *Advanced Gut & Microbiome Research*, *2024*(1), 4326438.
- Alqazlan, N., Alizadeh, M., Boodhoo, N., Taha-Abdelaziz, K., Nagy, E., Bridle, B., & Sharif, S. (2020). Probiotic Lactobacilli Limit Avian Influenza Virus Subtype H9N2 Replication in Chicken Cecal Tonsil Mononuclear Cells. *Vaccines*, *8*(4), Article 4. https://doi.org/10.3390/vaccines8040605
- Balouria, A., Deshmukh, S., Banga, H. S., Ahmad, A., Brar, R. S., & Sodhi, S. (2019). Early migration pattern of Avibacterium paragallinarum in the nasal passage of experimentally infected chicken and Japanese quail by immunohistochemistry. *Avian pathology*, *48*(2), 168-177.
- Chen, X., Ishfaq, M., Hu, F., Zheng, Q., Hu, X., Wang, J., & Huo, N. (2023). Bacillus subtilis KC1 prevents Mycoplasma gallisepticum-induced lung injury by enhancing intestinal Bifidobacterium animalis and regulating indole metabolism in chickens. *Poultry Science*, *102*(8), 102824. https://doi.org/10.1016/j.psj.2023.102824
- Guo, M., Liu, D., Chen, X., Wu, Y., & Zhang, X. (2022). Pathogenicity and innate response to Avibacterium paragallinarum in chickens. Poultry Science, 101(1), 101523.
- Hor, Y. Y., Lew, L. C., Lau, A. S. Y., Ong, J. S., Chuah, L. O., Lee, Y. Y., ... & Liong, M. T. (2018). Probiotic Lactobacillus casei Zhang (LCZ) alleviates respiratory, gastrointestinal & RBC abnormality via immuno-modulatory, anti-inflammatory & anti-oxidative actions. Journal of Functional Foods, 44, 235-245.
- Lutful Kabir, S. M. (2009). The role of probiotics in the poultry industry. International journal of molecular sciences, 10(8), 3531-3546.

References

- Khomayezi, R., & Adewole, D. (2022). Probiotics, prebiotics, and synbiotics: an overview of their delivery routes and effects on growth and health of broiler chickens. World's Poultry Science Journal, 78(1), 57-81.
- Krysiak, K., Konkol, D., & Korczyński, M. (2021). Overview of the Use of Probiotics in Poultry Production. Animals: An Open Access Journal from MDPI, 11(6), 1620. https://doi.org/10.3390/ani11061620

- Khomayezi, R., & Adewole, D. (2022). Probiotics, prebiotics, and synbiotics: an overview of their delivery routes and effects on growth and health of broiler chickens. World's Poultry Science Journal, 78(1), 57-81.
- Krysiak, K., Konkol, D., & Korczyński, M. (2021). Overview of the Use of Probiotics in Poultry Production. *Animals: An Open Access Journal from MDPI*, *11*(6), 1620. https://doi.org/10.3390/ani11061620

- Luan, S. J., Sun, Y. B., Wang, Y., Sa, R. N., & Zhang, H. F. (2019). Bacillus amyloliquefaciens spray improves the growth performance, immune status, and respiratory mucosal barrier in broiler chickens. *Poultry Science*, *98*(3), 1403–1409. https://doi.org/10.3382/ps/pey478
- Lutful Kabir, S. M. (2009). The Role of Probiotics in the Poultry Industry. *International Journal of Molecular Sciences*, *10*(8), 3531–3546. https://doi.org/10.3390/ijms10083531
- Morales-Erasto, V., Falconi-Agapito, F., Luna-Galaz, G. A., Saravia, L. E., Montalvan-Avalos, A., Soriano-Vargas E, E., & Fernández-Díaz, M. (2016). Coinfection of Avibacterium paragallinarum and Ornithobacterium rhinotracheale in Chickens from Peru. Avian Diseases, 60(1), 75–78. https://doi.org/10.1637/11265-082015-ResNote.1
- Oladokun, S., & Sharif, S. (2024). Exploring the complexities of poultry respiratory microbiota: Colonization, composition, and impact on health. Animal Microbiome, 6(1), 25. https://doi.org/10.1186/s42523-024-00308-5

References

- Nouri Gharajalar, S., Mirzai, P., Nofouzi, K., & Madadi, M. S. (2020). Immune enhancing effects of *Lactobacillus acidophilus* on Newcastle disease vaccination in chickens. *Comparative Immunology, Microbiology and Infectious Diseases*, *72*, 101520. https://doi.org/10.1016/j.cimid.2020.101520
- Rasaei, D., Hosseinian, S. A., Asasi, keramat, Shekarforoush, S. S., & Khodakaram-Tafti, A. (2023). The beneficial effects of spraying of probiotic *Bacillus* and *Lactobacillus* bacteria on broiler chickens experimentally infected with avian influenza virus H9N2. *Poultry Science*, *102*(7), 102669. https://doi.org/10.1016/j.psj.2023.102669
- Paudel, S., Ruhnau, D., Wernsdorf, P., Liebhart, D., Hess, M., & Hess, C. (2017). Presence of Avibacterium paragallinarum and Histopathologic Lesions Corresponds with Clinical Signs in a Co-infection Model with Gallibacterium anatis. Avian Diseases, 61(3), 335–340. https://doi.org/10.1637/11609-021317-RegR
- Peek, H. W., & Landman, W. J. M. (2011). Coccidiosis in poultry: Anticoccidial products, vaccines and other prevention strategies. Veterinary Quarterly, 31(3), 143–161. https://doi.org/10.1080/01652176.2011.605247
- Redweik, G. A. J., Jochum, J., & Mellata, M. (2020). Live Bacterial Prophylactics in Modern Poultry. *Frontiers in Veterinary Science*, *7*. https://www.frontiersin.org/articles/10.3389/fvets.2020.592312

References

- Talebi, A., Amani, A., Pourmahmod, M., Saghaei, P., & Rezaie, R. (2015). Synbiotic enhances immune responses against infectious bronchitis, infectious bursal disease, Newcastle disease and avian influenza in broiler chickens. *Veterinary Research Forum*, *6*(3), 191–197.
- Tiwari, G., Tiwari, R., Pandey, S., & Pandey, P. (2012). Promising future of probiotics for human health: Current scenario. Chronicles of Young Scientists, 3(1), 17-17.
- Wang, J., Ishfaq, M., Fan, Q., Chen, C., & Li, J. (2020). A respiratory commensal bacterium acts as a risk factor for Mycoplasma gallisepticum infection in chickens. *Veterinary Immunology and Immunopathology*, *230*, 110127. https://doi.org/10.1016/j.vetimm.2020.110127
- Wang, J., Ishfaq, M., & Li, J. (2021). *Lactobacillus salivarius* ameliorated *Mycoplasma gallisepticum*-induced inflammatory injury and secondary *Escherichia coli* infection in chickens: Involvement of intestinal microbiota. *Veterinary Immunology and Immunopathology*, *233*, 110192. https://doi.org/10.1016/j.vetimm.2021.110192
- Wu, Y., Wang, Y., Yang, H., Li, Q., Gong, X., Zhang, G., & Zhu, K. (2021). Resident bacteria contribute to opportunistic infections of the respiratory tract. *PLoS Pathogens*, *17*(3), e1009436.
- Zhang, R., Zhang, L., Li, P., Pang, K., Liu, H., & Tian, L. (2023). Epithelial barrier in the nasal mucosa, related risk factors and diseases. *International Archives of Allergy and Immunology*, *184*(5), 481-501.
- Zhu, J., Chen, Y., Wu, Y., Wang, Y., & Zhu, K. (2022). Commensal bacteria contribute to the growth of multidrug-resistant Avibacterium paragallinarum in chickens. *Frontiers in Microbiology*, *13*, 1010584.