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Applied Research Note: Bacterial profile in the environment of an egg-producing farm in Southern Brazil

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Primary Audience: Researchers, poultry microbiologists, poultry farmers, poultry industry

SUMMARY

This study aims to characterize the environmental microbiota of an egg-producing farm situated in Rio Grande do Sul, Southern Brazil, spanning from the chick to the laying hen stages and encompassing the treatment of carcasses and manure. Metataxonomy analyses reveal the continuity of bacterial diversity across the production stages (chick, pullet, and laying hen). The presence of *Fusobacteriota* and *Cyanobacteria* in poultry environments before any manure or carcass treatments (named pre-treatment samples) are identified as indicative phyla markers for healthy animals. Nonetheless, alterations in the bacterial communities emerge during the treatment of manure and carcasses (treatment samples), revealing an increased abundance of *Halanaerobiaeota*. In summary, the study underscores the key phyla influencing the entire environment of the egg production process on a farm in South Brazil. Although our data is from a specific farm, it provides insights for a more robust and representative study of the egg chain.

Key words: poultry, laying hen, composting, 16S-rDNA analysis

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DESCRIPTION OF PROBLEM

Microbiome studies to understand bacterial population dynamics in the environment have gained visibility over the last few years (He et al., 2019; Tyrrell et al., 2023). However, there is scant research about the composition of the bacterial communities in the environment of egg-producing farms.

Animal production farms treat manure, commonly used as fertilizer, creating contact with soil or water. This contact may influence ecosystems, posing risks to microbial balance, such as water contamination by pathogens (Mulder et al., 2020; Gržinić et al., 2023). Consequently, this study aimed to explore the dynamics of bacterial communities' composition throughout the layer hen production chain, including carcass and manure treatment.

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MATERIALS AND METHODS

Study Design

Sampling was carried out in April 2022 (in the autumn) in an intensive egg-production farm in Rio Grande do Sul state (Southern Brazil) [29°32′35.512″S 51°4′30.262″W]. A total of 90,000 birds (8,000 chicks, 17,000 pullets, and 65,000 laying hens), belonging to one of the following breeds: HyLine W-36 (43,875 laying hens), HyLine Brown (14,625 laying hens), and Dekalb (6,500 laying hens) were kept on the farm. The chick phase lasted from d 1 to d 40, while the pullet phase extended from d 41 to d 115. The laying hens were kept in production for 100 wk on average and kept in 5 houses with a pyramid system and one house with a vertical system. Each breed was placed in a separate house. Further, the farm had a monthly production of approximately 1.8 million eggs, with an average of 492 eggs per bird for HyLine W-36, 500 for HyLine Brown, and 486 for Dekalb.

The poultry diet main ingredients were corn, soybean bran, canola bran, wheat bran, limestone, vitamins, minerals, probiotics, and organic acids. The diet formulation varied according to breed, age, and production phase. Consequently, 3 diets were formulated for the chick and pullet phases and three for the laying hen phase. Antibiotics were not used for prophylactic purposes or as growth promoters. Moreover, facilities were cleaned and disinfected after discarding each flock, followed by a 15-d down time.

Chicks, pullets, and laying hens were placed in separate houses by the farm administration, with chicks kept on wood shavings litter. In contrast, pullets and laying hens were kept in conventional cages (375 cm^2/animal). Further, laying hens had battery cages, which were organized in a vertical or pyramidal layout. Additionally, the vertical system was equipped with a manure belt for excreta removal, while the pyramidal system allowed the mechanical removal of accumulated manure at the end of each week. The manure from all the animals was deposited in a composting shed, generating fertilizer after 120 d. Additionally, any carcasses were composted in boxes for 120 d. Notably, the accumulated average mortality rate per batch was 10% without the occurrence of disease outbreaks.

Environmental samples were collected for each production phase and composting (Table 1). One sterile swab was used for each area measuring 15 cm^2 from the bottom of the cages, and the floor of the laying hen, pullet, and chick houses were sampled and placed directly in PowerBead Tubes of the DNeasy PowerSoil kit (Qiagen, Hilden, Germany). The composting process samples (manure and carcass) and manure samples were collected using sterile conical tubes (20 g). Given

Table 1. Samples collected in the studied egg-producing farm.

Sample ID	Stage	Environmental sampled	Group
CF	Chick	Floor	Pre-treatment
CP	Pullet	Vertical battery cage	Pre-treatment
MP	Pullet	Vertical battery manure-belt	Pre-treatment
CLH	Laying hen	Pyramidal battery cage	Pre-treatment
FLH	Laying hen	Pyramidal battery house floor	Pre-treatment
MLH_7days	Laying hen	Pyramidal battery manure (7 d)	Pre-treatment
MLH	Laying hen	Vertical battery manure-belt	Pre-treatment
Carcass_10days	Carcass composting	Box content (10 d)	Treatment
Carcass_40days	Carcass composting	Box content (40 d)	Treatment
Manure_10days	Manure composting	Contents of the treatment shed (10 d)	Treatment
Manure_40days	Manure composting	Contents of the treatment shed (40 d)	Treatment

Pre-treatment group: samples in shed before the entry composting; Treatment group: samples in processes of composting. CF: chick floor, CP: cage pullet, MP: manure pullet, CLH: cage laying hen, FLH: house floor laying hen, MLH_7days: the accumulated manure from laying hens in the pyramidal system over 7 d, MLH: manure laying hen in vertical battery, Carcass_10days: carcass composting in 10 d, Carcass_40days: carcass composting in 40 d, Manure_10days: manure composting in 10 d, Manure_40days: manure composting in 40 d. Chick phase: d 1 to d 40; Pullet phase: d 41 to d 115; Laying hen phase: d 116 to 100 wk.

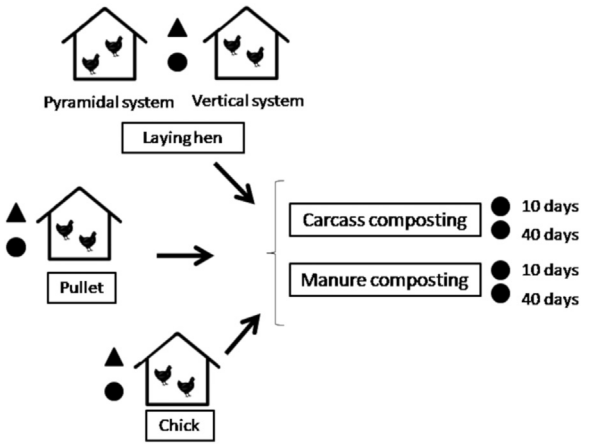

Figure 1. Schematic representation of the samplings conducted at the farm. Triangles represent swab collections, and circles represent content collections (manure and the both composting).

that the composting of animal manure and carcasses occurred in a single location, the samples were divided into two groups (pre-treatment and treatment), where the treatment group was linked to the composting process (Figure 1 and Table 1).

According to the literature, there is an increase in *Firmicutes, Actinobacteria*, and *Bacteroidetes* and a decrease in *Proteobacteria* in composted swine manure (Chang et al., 2024). Therefore, we expect that the same conditions will be found in composted manure and carcasses of laying hens.

Structure Analysis of Bacterial Community

Samples were kept at 4°C until processing, and DNA extraction was performed using the DNeasy PowerSoil kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Additionally, a blank sample, represented by the DNA extracted from ultra-pure water (negative control), was prepared for further discarded amplicon sequence variants associated with the blank. Further, polymerase chain reaction of the V4 region of the *16S-rDNA* gene and sequencing were performed using MiSeq v2 500 kit-cycles in a MiSeq System (Illumina Inc., San Diego, CA).

Moreover, after checking raw read quality using FastQC software (v0.11.4), single-end FASTQ files were imported into Quantitative Insights into Microbial Ecology version 2 (**QIIME 2**). Thereafter, taxonomic analysis was performed using the DADA2 package with SILVA 138 database. Notably, in the R 4.1.1 software, microbiome, phyloseq (v1.38.0), and microDecon packages were used. Eukaryote, archaea, chloroplast, mitochondria, unknown classification, and taxa with a confidence level < 0.8 were removed. Further, beta diversity (Unweighted UniFrac and PERMANOVA with a permutation number of 999) was calculated, and differential abundance was evaluated using microeco (v0.7.1).

Metabolic pathways were evaluated through principal component analysis (**PCA**) constructed from PICRUSt2 (v2.0.0-b.2) and STAMP (v2.1.3). Additionally, unclassified reads were removed and White's nonparametric *t*test ($P < 0.05$) was used. All sequencing data were submitted to the NCBI Sequence Read Archive (SRA) database under BioProject accession PRJNA1054821.

RESULTS AND DISCUSSION

To comprehend the dynamics of the bacterial community within the environment of an egg-producing farm, diversity analyses were conducted. Beta diversity analysis revealed clustering in the pretreatment and treatment groups (PERMANOVA: $P = 0.006$; Adonis: $R^2 = 0.17$) (Figure 2A). Interestingly, the manure sample of laying hens (MLH_7d) fell within the intersection of the analyzed groups (Figure 2A), which may be related to the short time since manure deposition, representing the beginning of the composting process (Agnew and Leonard, 2003).

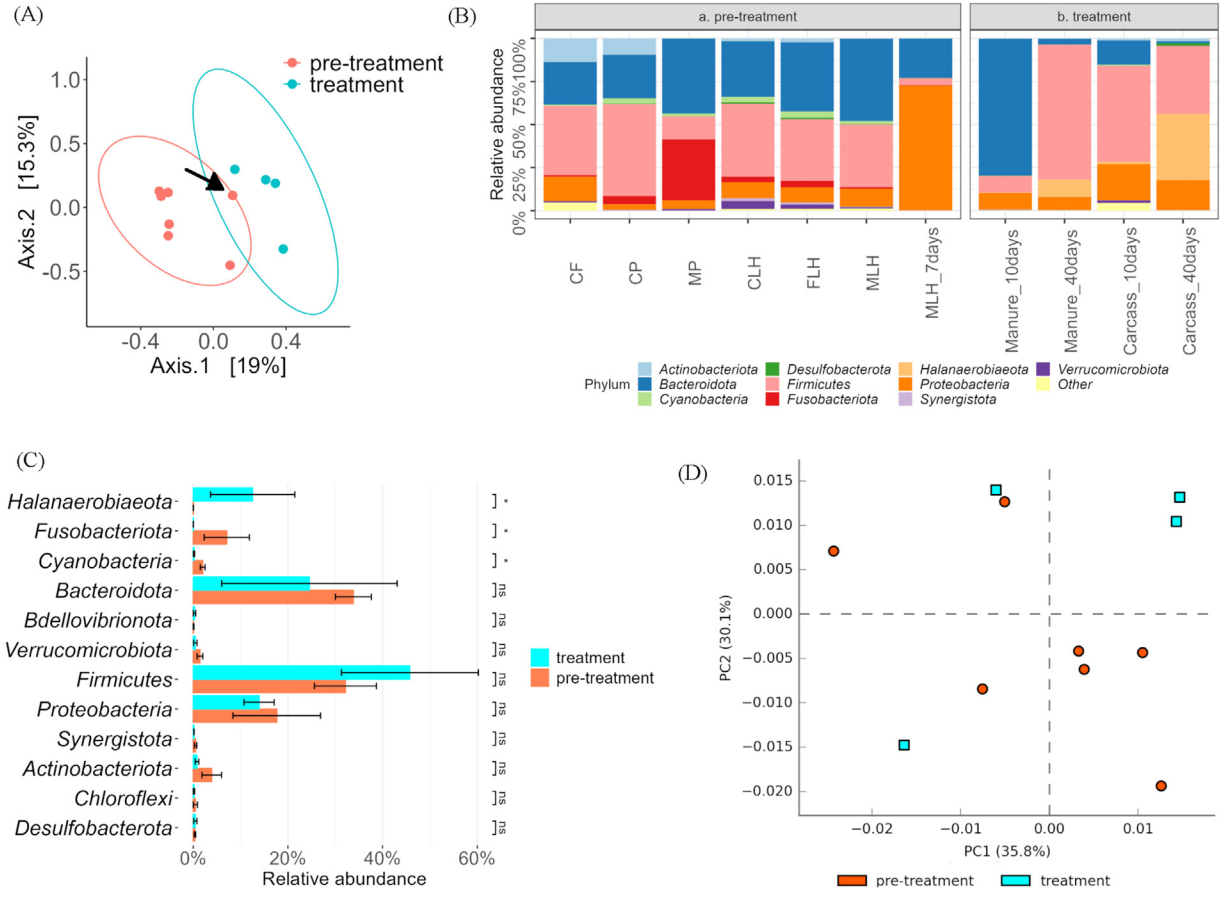

Figure 2. Bacterial community dynamic in an egg-production farm. (A) Unweighted Unifrac beta diversity analysis (PERMANOVA: P = 0.006). The arrow represents the MLH_7days sample. (B) Relative abundance at phylum level. (C) Differential abundance between pre-treatment and treatment groups. (D) Principal component analysis (**PCA**) for metabolites pathways in pre-treatment and treatment groups.*: Wilcoxon rank-sum test with $P < 0.05$. ns: nonstatistical significance.

Regarding relative abundance at the phylum level (Figure 2B), the pre-treatment bacterial composition maintained a uniform diversity, with a high relative abundance of *Actinobacteriota* in the chick floor (**CF**). Moreover, in the pullet phase, there was a high relative abundance of *Fusobacteriota* in manure-belt pullet (**MBP**) and cage pullet (**CP**). Further, in the layer hen phase, there was a high relative abundance of *Desulfobacterota* and *Verrucomicrobiota* in cage-laying hens (**CLH**) and house floor-laying hens (**FLH**). The samples CF, CP, MP, CLH, FLH, and MLH presented the same bacterial profile found in a study of laying hen manure samples (Mazhar et al., 2021), showing that manure content is important to the formation of the bacterial profile present in the farm environment.

The bacterial composition of MLH_7d, with a high amount of *Proteobacteria*, was different from the composition of other samples (Figure 2B). This phylum is present in different environments, such as soil (Spain et al., 2009) and in the gut of animals (Mazhar et al., 2021). Considering that MLH_7d could initiate composting processes, elevated levels of *Proteobacteria* might be associated with the mesophilic stage, followed by a subsequent decline during the thermophilic stage (Biyada et al., 2021).

The taxonomic profile of the bacterial community displayed differences between the pre-treatment and treatment groups (Figures 2A and 2B). In the treatment group, all the samples (manure and carcass) had a high concentration of *Bacteroidota, Firmicutes, Halanaerobiaeota,* and *Proteobacteria*. The presence of the phyla *Proteobacteria, Firmicutes*, and *Bacteroidota* was previously reported in composted swine manure (Xu et al., 2023a). Further, there were high relative abundance values of *Firmicutes* in soils with copper and zinc as sulfate or nitrate salts (Fortunato et al., 2021) and in soils with Pb, Cd, and Zn contamination (Fajardo et al., 2019). Thus, the introduction of *Firmicutes* bacteria through treated waste used for agricultural fertilization may indicate the presence of heavy metals in the soil.

Interestingly, the proportion of *Bacteroidota* decreased while that of *Halanaerobiaeota* increased in 40 d in the carcass and manure composting samples (Figure 2B). *Bacteroidota* is positively correlated with the presence of nitrate and ammonium (Ma et al., 2023). Consequently, *Bacteroidota* decrease may be related to the reduction of these compounds due to the composting process. Furthermore, *Bacteroidota* may be directly associated with protein concentration (Perman et al., 2022), which would justify the decrease in relative abundance due to the low level of available protein from carcass degradation.

There was a high relative abundance of *Cyanobacteria* and *Fusobacteriota* in the pre-treatment group ($P < 0.05$), while *Halanaerobiaeota* was abundant in the treatment group ($P < 0.05$) (Figure 2C). Further, in a previous study of layer hen fecal microbiome, there was a high abundance of *Firmicutes, Bacteroides*, and *Fusobacteria* in the high-laying group and a high abundance of the phyla *Actinobacteria, Cyanobacteria*, and *Proteobacteria* in the low-laying group (Elokil et al., 2020), potentially indicating phyla related to animal performance.

Regarding *Halanaerobiaeota,* an increased abundance in the presence of magnesite added to swine manure compost was observed, with a positive correlation to temperature, C/N ratio, pH, and urease (Xu et al., 2023b). Moreover, *Halanaerobiaeota* may be involved in fermentation processes, as observed in the case of vegetables and dairy products (Liang et al., 2023), potentially leading to an increase in relative abundance during composting.

Consequently, *Cyanobacteria* and *Fusobacteriota* composition can be explored before the introduction of manure into the treatment process, thereby contributing to an understanding of animal performance. Furthermore, bacteria belonging to the phylum *Halanaerobiaeota* can serve as physicochemical markers of processes occurring during the treatment of poultry manure.

In the analyses of metabolic routes, samples from the pre-treatment group showed a tendency to cluster (Figure 2D). The configuration of PCA may be related to the bacterial composition of the samples, which are similar in the pre-treatment group (Figure 2B). Regarding the metabolic pathways that showed statistical differences ($P < 0.05$) between groups, the cell structure biosynthesis (PWY0-1586) and the generation of precursor metabolites and energy (PWY-7254) were prominent in the treatment group (Figure 3).

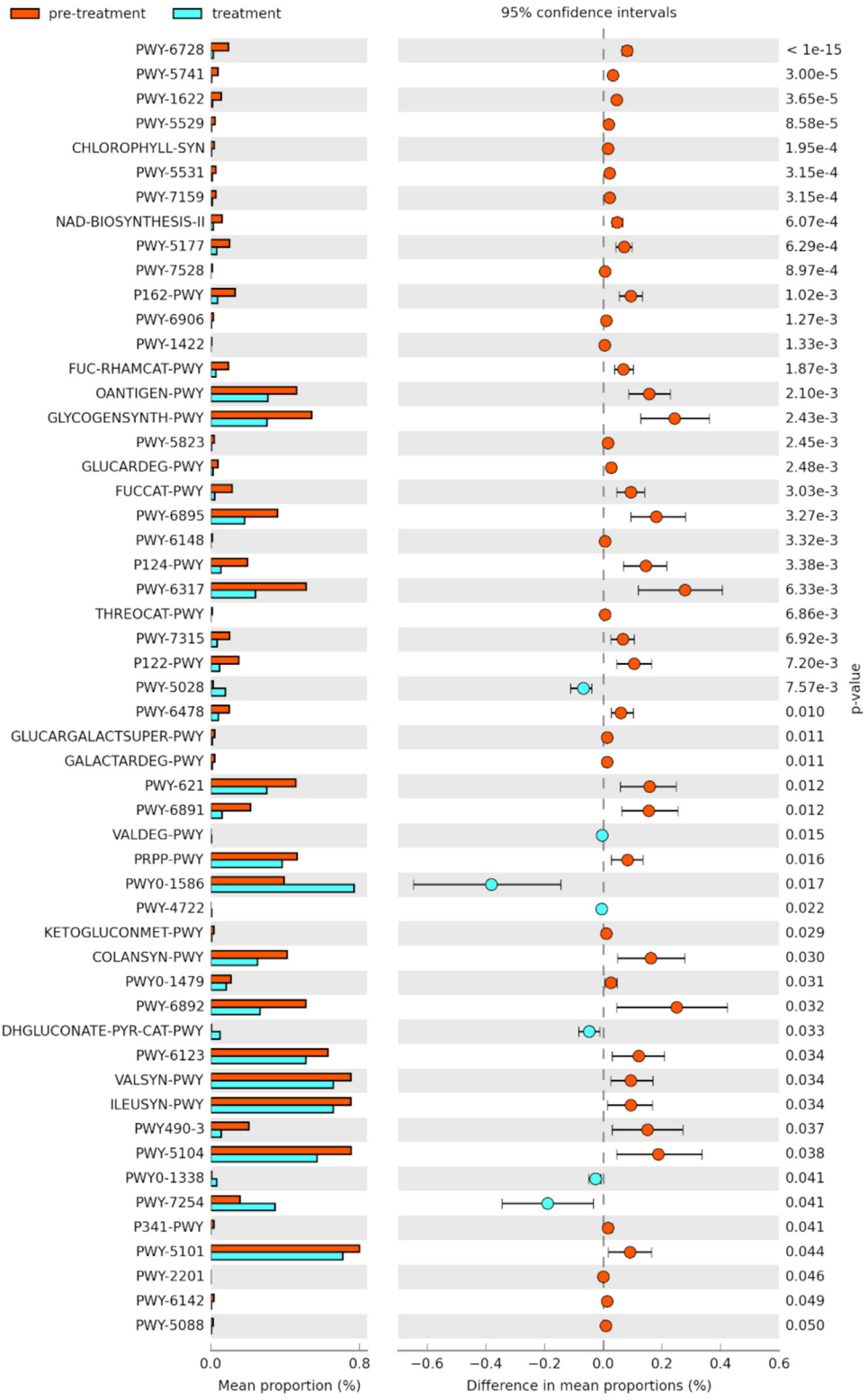

Figure 3. Differential metabolic pathways between pre-treatment and treatment groups.

CONCLUSIONS AND APPLICATIONS

This study illustrates the consistent preservation of the bacterial community profile throughout the laying hens production cycle in the analyzed farm. However, notable variations emerged when examining the composting of carcasses and manure, revealing distinct changes compared to other samples (CF, CP, MP, CLH, FLH, and MLH). Notably, contrary to our initial hypothesis, there was a high occurrence of *Fusobacteriota* and *Cyanobacteria* in samples from the pre-treatment group and a high occurrence of *Halanaerobiaeota* in the samples from the treatment group.

Given the specificity of the sampling time and that only one farm was involved, caution is warranted in making broad generalizations using our results. Nevertheless, the phyla (*Cyanobacteria, Fusobacteriota,* and *Halanaerobiaeota*) identified in this study can guide future research about the bacterial composition of a commercial poultry farm and waste treatment processes. There is an opportunity to conduct further studies aiming to unravel the dynamics of bacterial communities in the entire egg production process up to the point of human consumption. Such studies could significantly contribute to our understanding of the processes involved in the complex egg production ecosystem.

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DISCLOSURES

The authors declare no conflicts of interest.

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