

Water-wash or Barn Cleaning with Disinfectants: Which Is Better for the Broiler Gut Microbiota and Gut Health?

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About Microbiome Studies...



- Microbiome refers to the combined genetic material of the microorganisms in a particular environment.
- About 40% of the gut microbiome remains
- High-throughput technologies: 16S rRNA amplicon sequencing, shotgun metagenomic sequencing, RNA-Seq...



- ❑ Mass Production:
~ 14,000 birds per barn

Intro: Modern Broilers



Gallus sooneratti
(Grey Jungle Fowl)



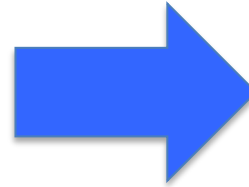
Gallus lafayettei
(Ceylon Jungle Fowl)



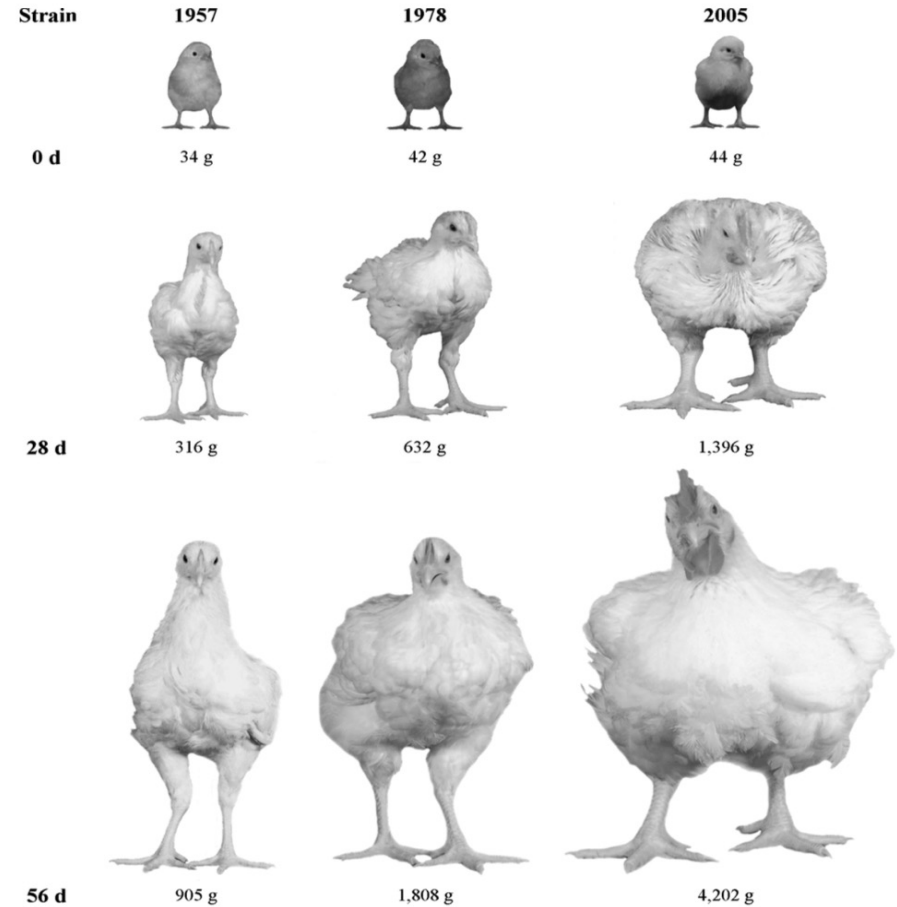
Gallus varius
(Green Jungle Fowl)



Gallus gallus
(Red Jungle Fowl)

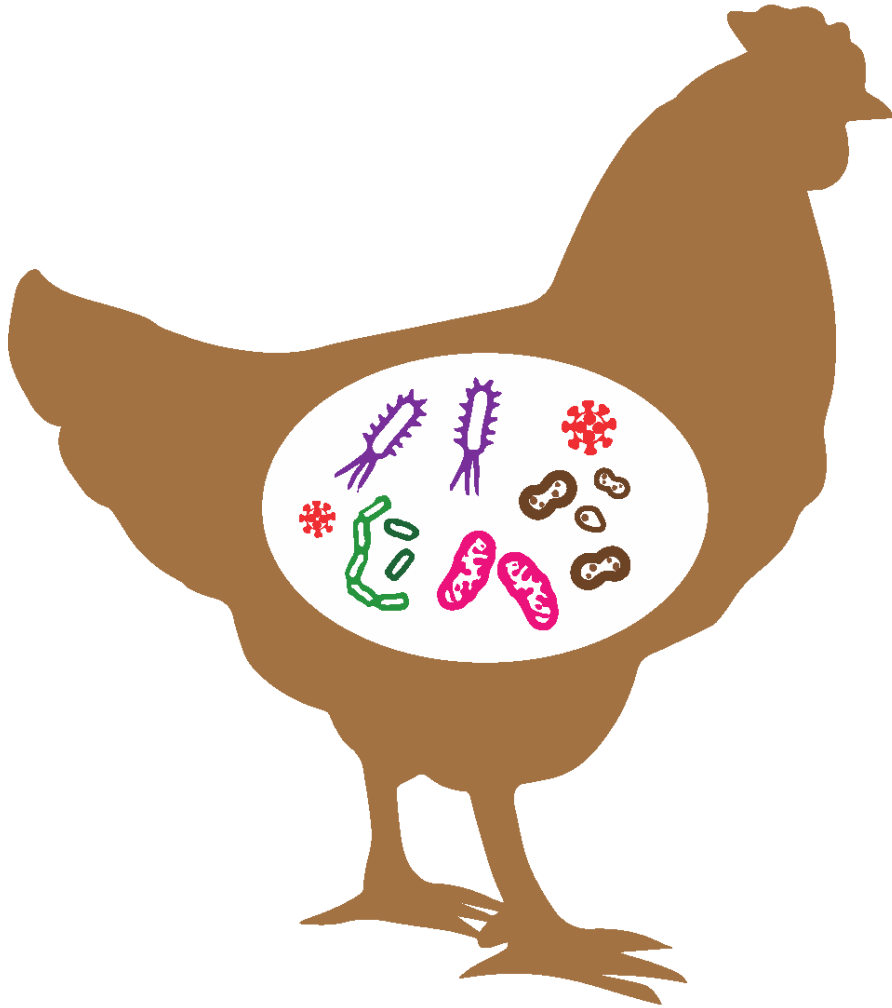


(Price et al., 2015)

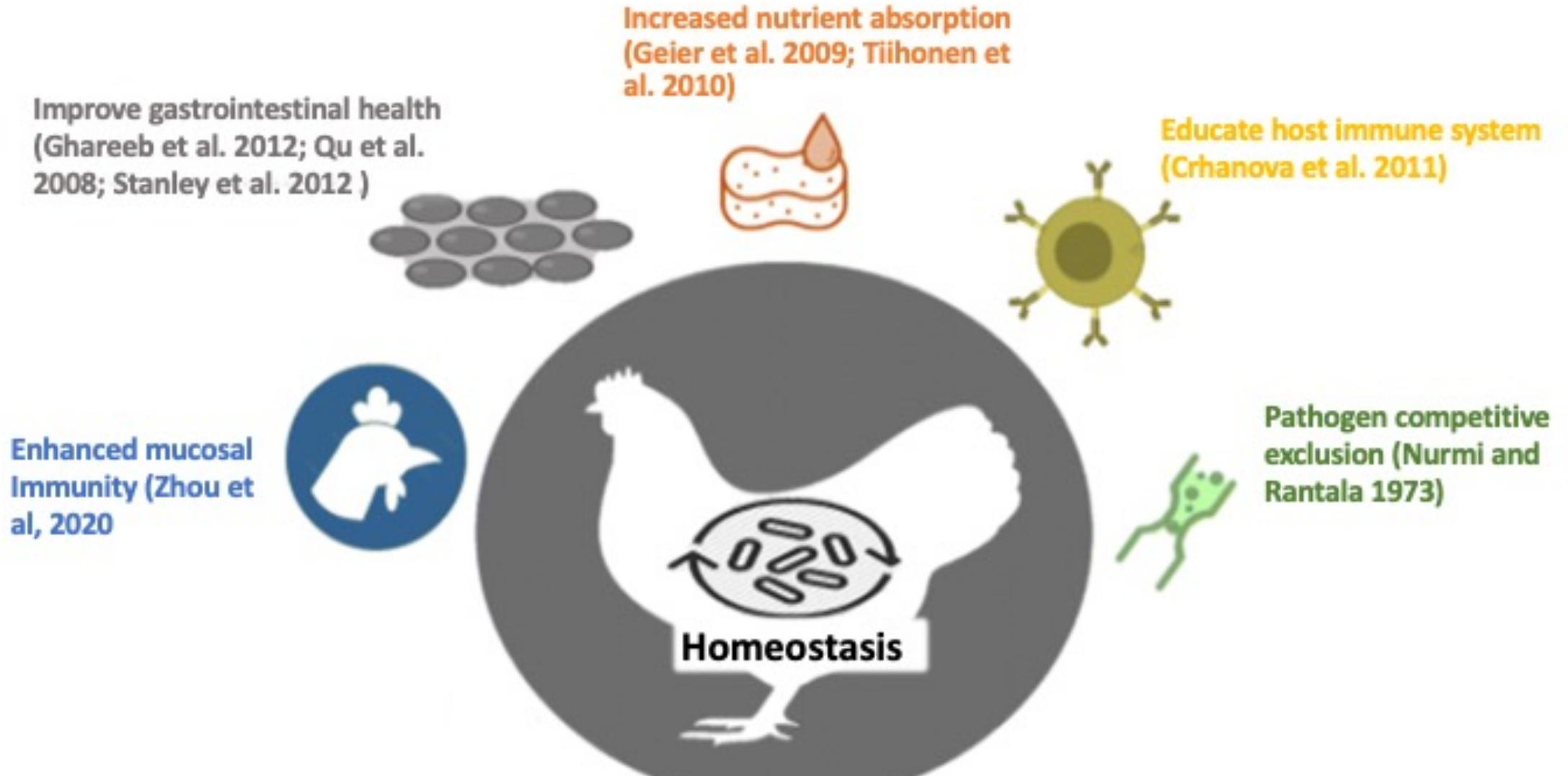


Gallus gallus domesticus
(Domesticated chickens)

(Zuidhof et al., 2014)

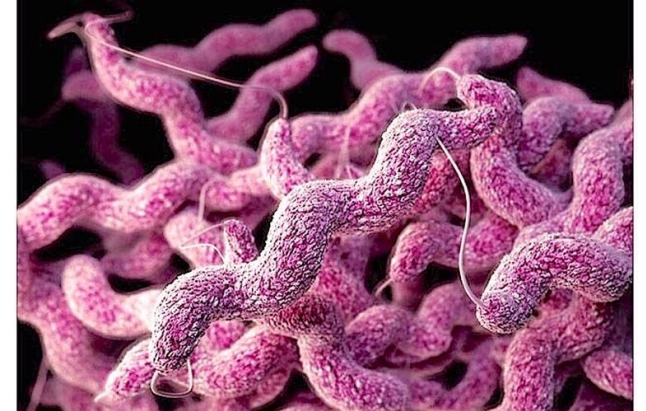


- Microbe-host co-evolution
- Commensals are involved in host physiological functions
- Hosts offers immune tolerance to commensals



Intro: Some Challenges

- Pathogens affecting chicken and human health (e.g. *Clostridium perfringens*, *Salmonella*, *Campylobacter*, etc.)



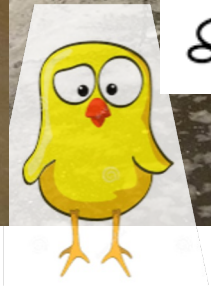
- Increased opposition to antibiotic growth promoter use

- Bedding materials significantly influenced chicken gut microbiota (Torek et al., 2009)
- Recycled litter significantly increased cecal (Torok et al., 2009; Wang et al., 2016) and jejunal (Cressman et al. 2010) microbial abundance and diversity in young chickens
- *Faecalibacterium prausnitizii* thrived in mature birds with recycled litter (Wang et al., 2016)
- Recycled litter increased pro-inflammatory cytokine expression in the chicken gut (Shanmugasundaram et al., 2012)

Water-wash (WW) VS. Full disinfection (FD)



Full disinfection
Or
Water wash?



Objectives



Determine the effect of barn sanitation practices on chicken performance, host gut microbiota, pathogen abundance, and microbial functionalities.

Barn Sanitation Design + Schedule

	Barn A	Barn B	Barn C	Barn D	Barn E	Barn F	Barn G
Cycle 1	FD	FD	FD	FD	FD	WW	WW
Cycle 2	FD	FD	WW	WW	FD	WW	WW
Cycle 3	WW	WW	WW	WW	WW	FD	FD
Cycle 4	WW	WW	FD	FD	WW	FD	FD

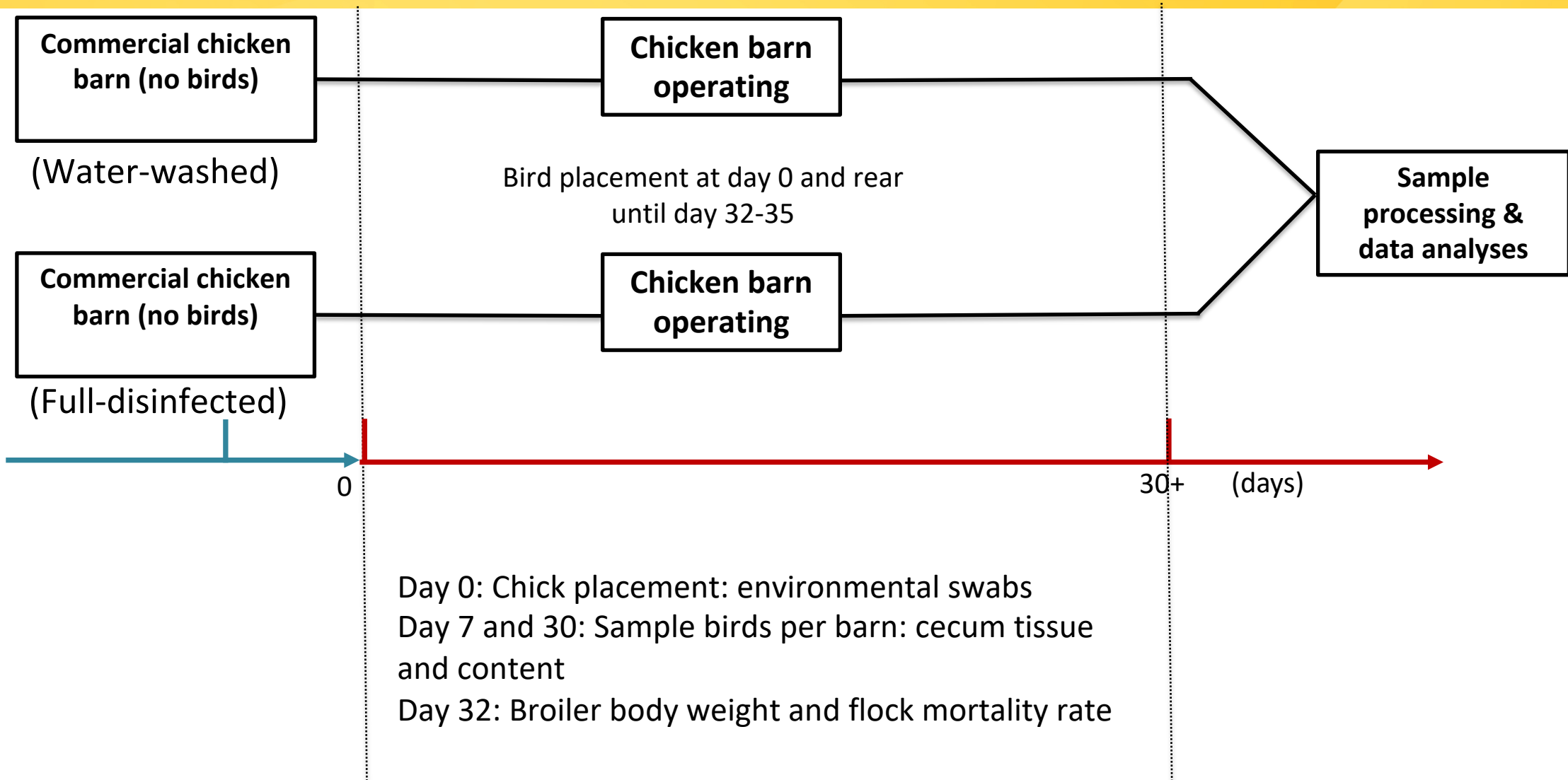
- 14 Water-Wash (WW) flocks vs. 14 Full Disinfection (FD) flocks

- Full disinfection:
- Completely remove manure, litter, and all organic compounds
- Cover all surfaces with an alkaline foam (NaOH, 2-(2-2-butoxyethoxy)ethanol, sodium laureth sulfate, tetrasodium EDTA), followed by high-pressure then low-pressure water rinse
- Cover all surfaces with an acidic foam (Glutaraldehyde, benzalkonium chloride and formic acid), followed by high-pressure water
- Fresh litter replacement

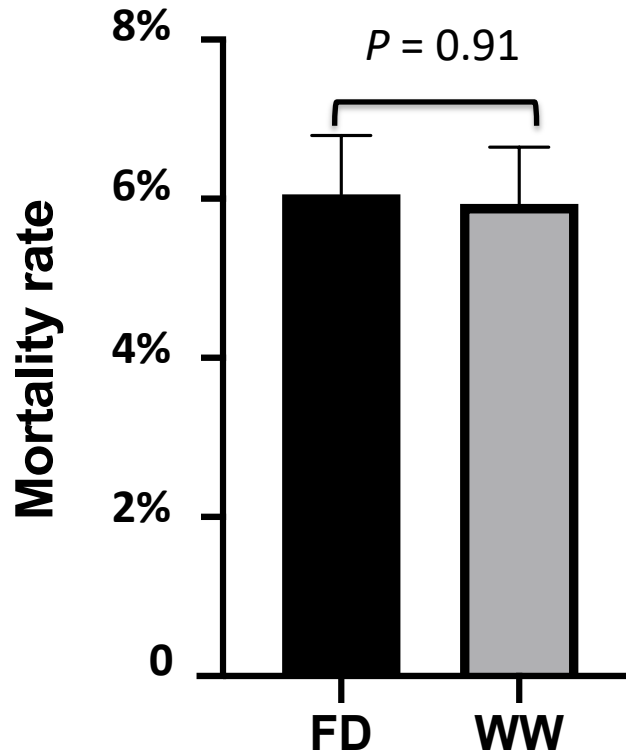
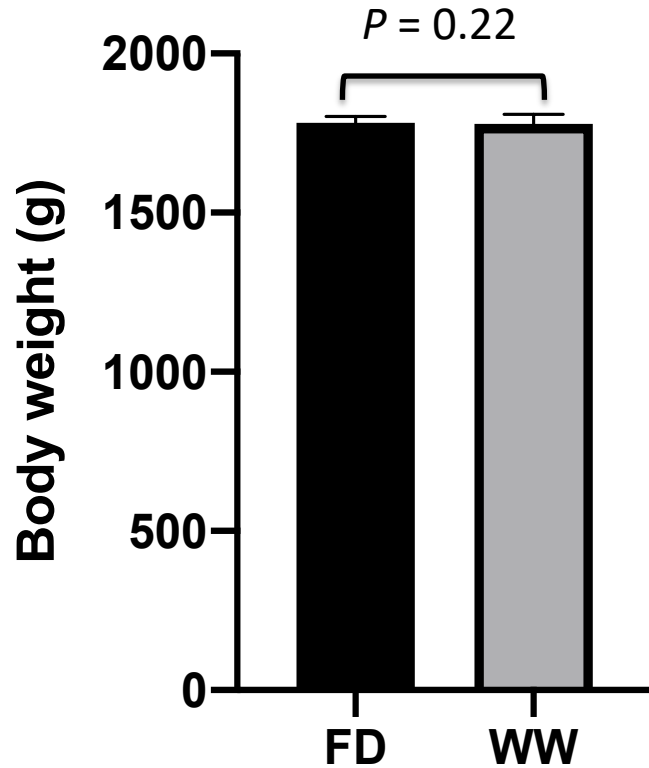
VS.

- Water-wash:
- Remove used litter and manure
- Wash all surfaces with water
- Fresh litter replacement

Animal Management and Sampling

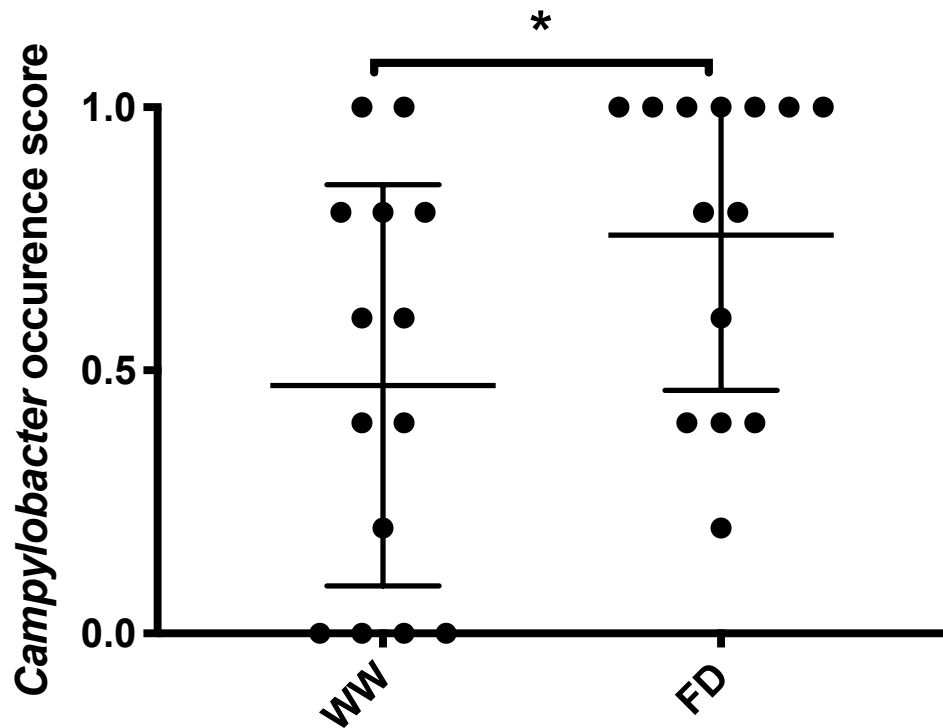


Result: Flock Performance



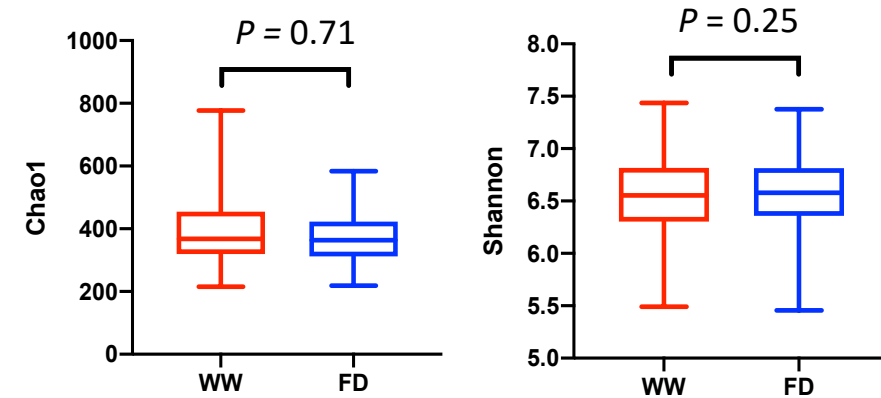
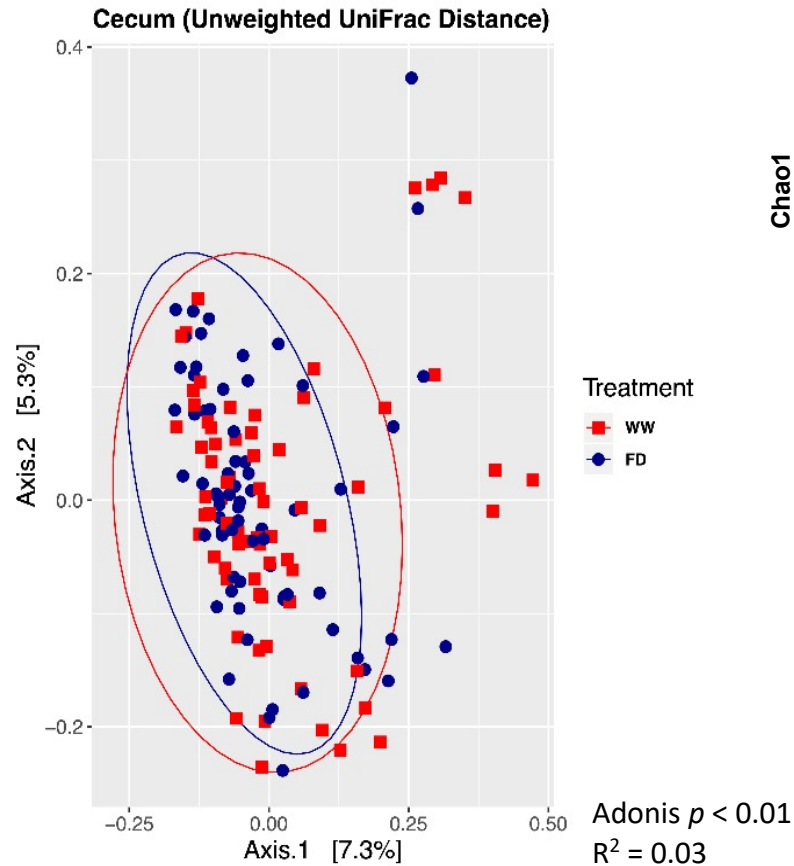
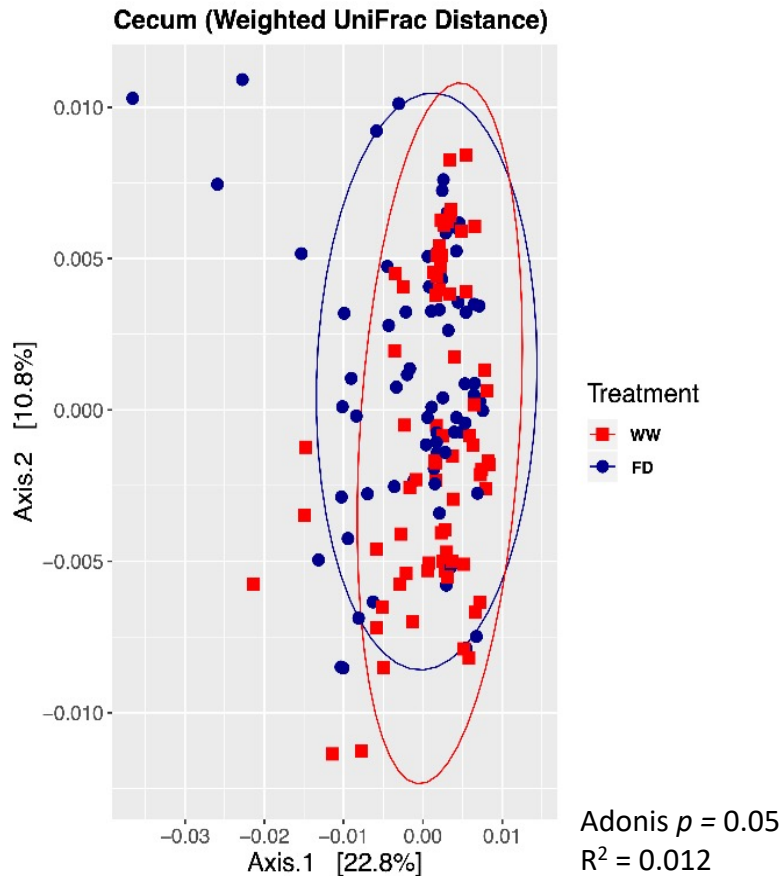
- Flock mean body weight at day 32 (FD: 1782 ± 30.09 g, WW: 1780 ± 20.59 g, mean ± SEM)
- Flock mean mortality rate at day 32 (FD: 6.1% ± 0.74%, WW: 5.9 ± 0.71%, mean ± SEM)
- Significant differences was determined by unpaired student's t-test

Result: Pathogen Enrichment



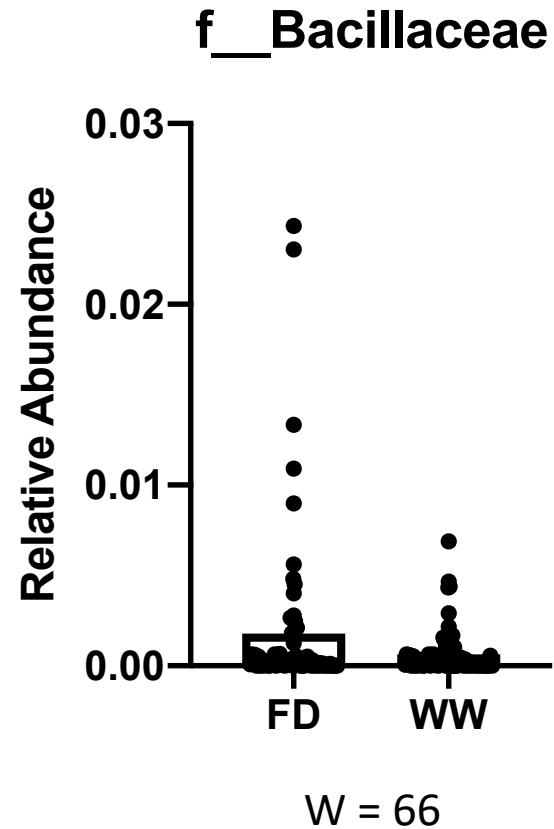
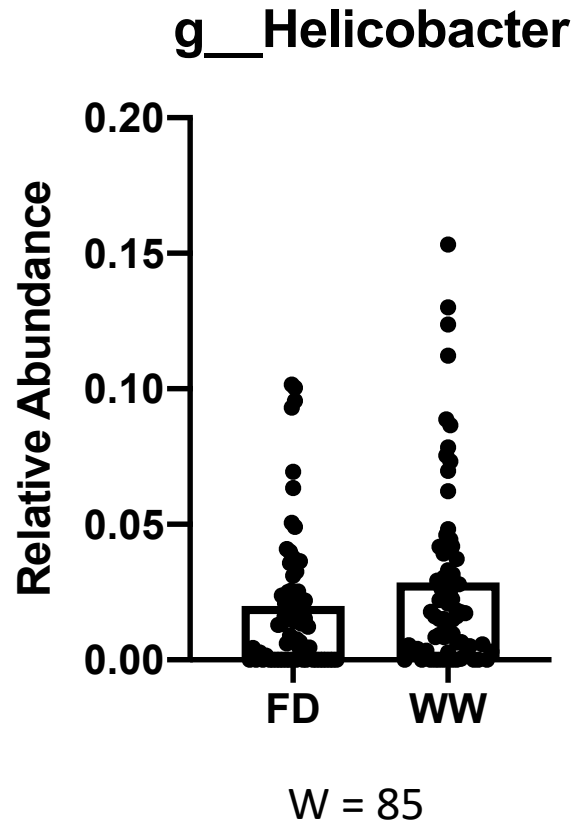
- Occurrence score = number of pathogen positive birds/ total number of birds sampled per barn
- No birds were found *Salmonella* positive
- At the end of the cycle, the WW group had less *Campylobacter* occurrence
- Significant differences was determined by unpaired student's t-test

Result: Chicken Microbiome α and β diversity



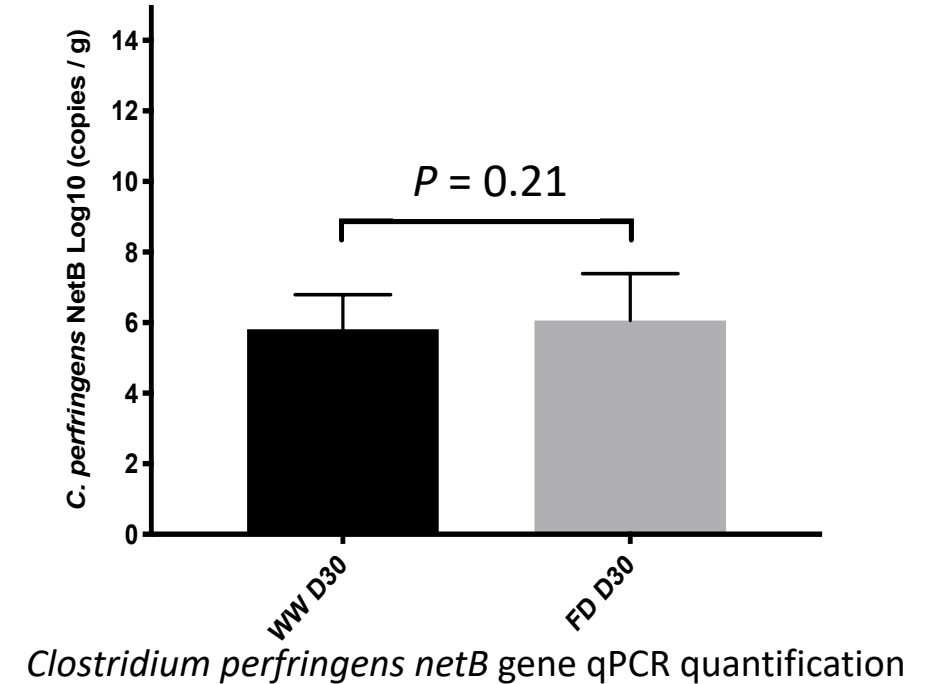
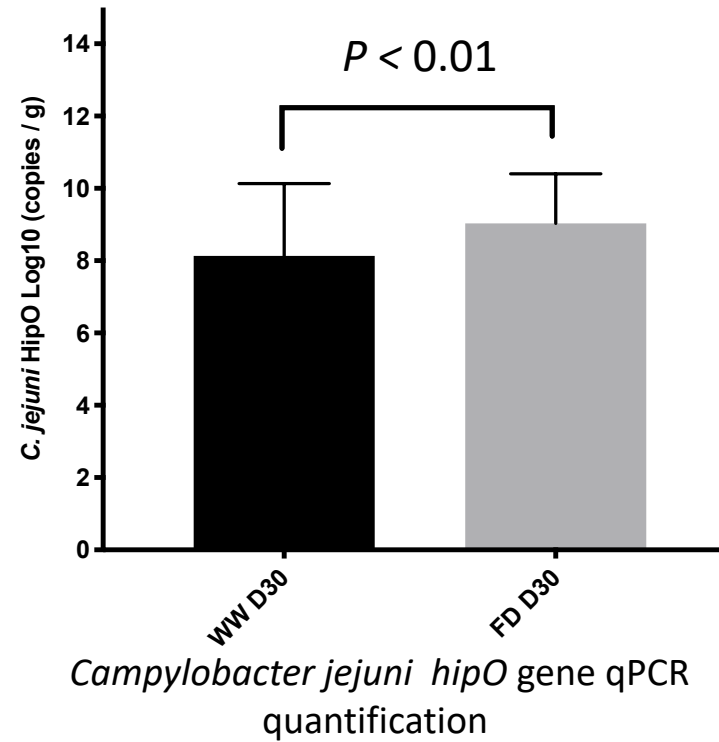
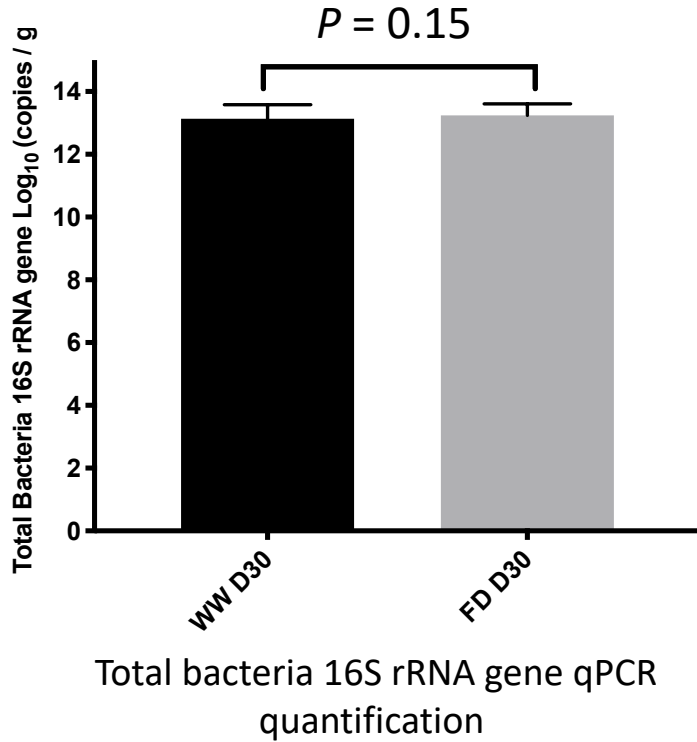
- 16S rRNA sequencing targeting V3-V4 region
- β diversity significance determined by PERMANOVA test
- α diversity significance determined by Kruskal-Wallis test

Result: Differentiated Taxa



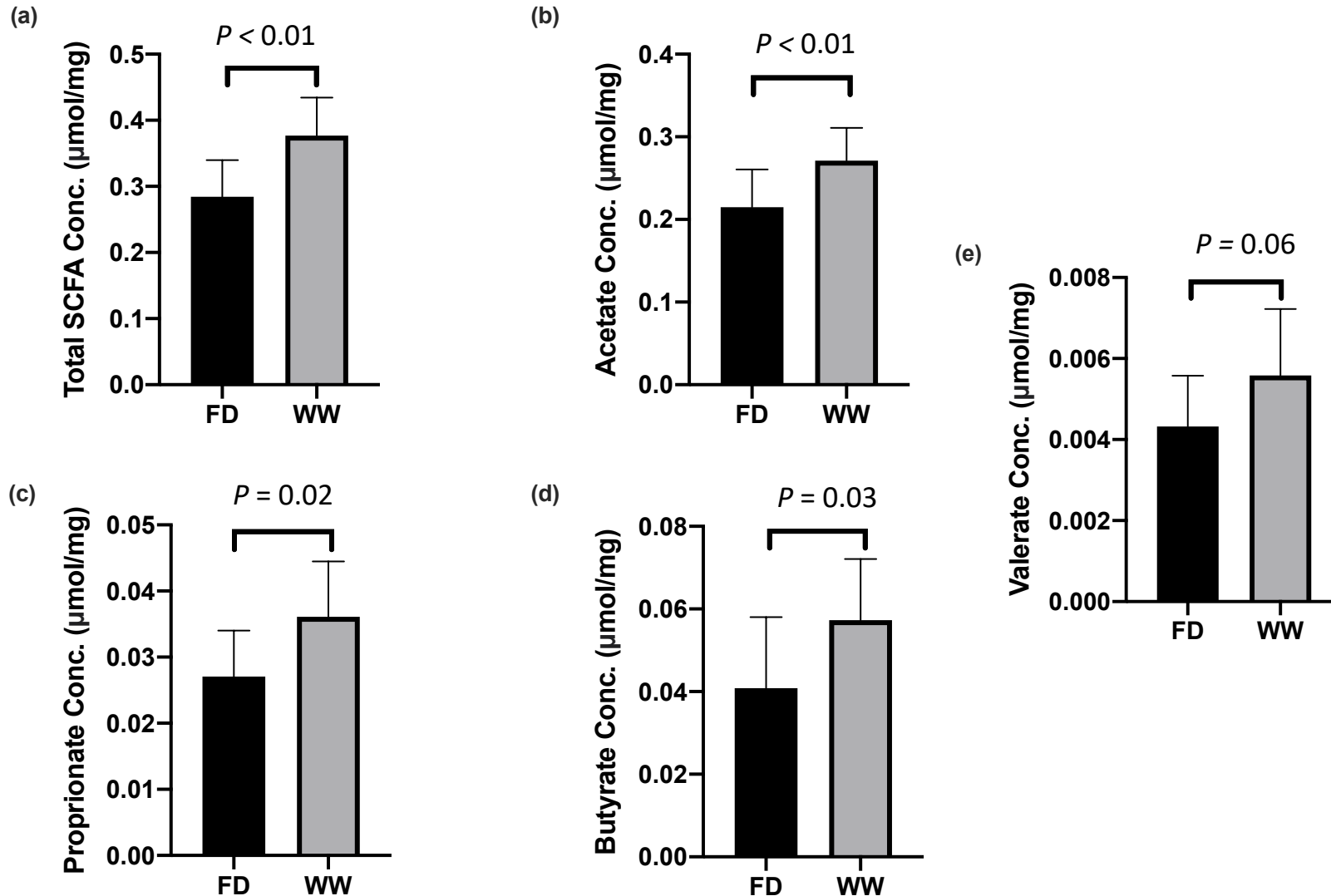
- Analysis of composition of microbiomes (ANCOM) identified 2 taxa had different relative abundance

Result: Pathogen-qPCR Assay



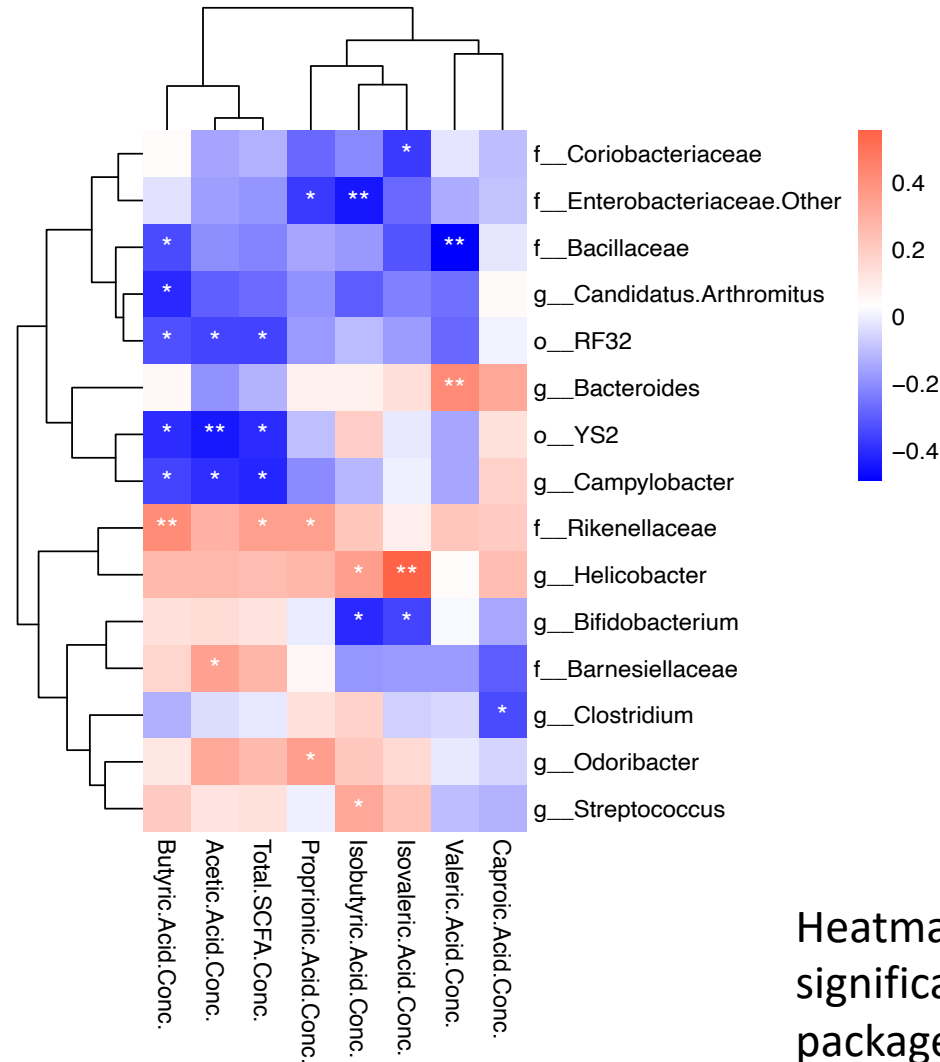
- qPCR targeting bacteria universal and species-specific gene
- Significant differences determined by unpaired student's t-test

Result: Cecal Short-chain Fatty Acids (SCFAs)



- SCFA concentration measured by gas chromatography
- Significant differences determined by unpaired student's t-test

Results: Gut Microbe-SCFA correlations



Heatmap was generated by R package corrplot, significant differences were determined by R package psych (*, $p < 0.05$; **, $p < 0.01$)

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The Use of Disinfectant in Barn Cleaning Alters Microbial Composition and Increases Carriage of *Campylobacter jejuni* in Broiler Chickens

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- How does the altered microbiota affect the cecal microbial functionality?

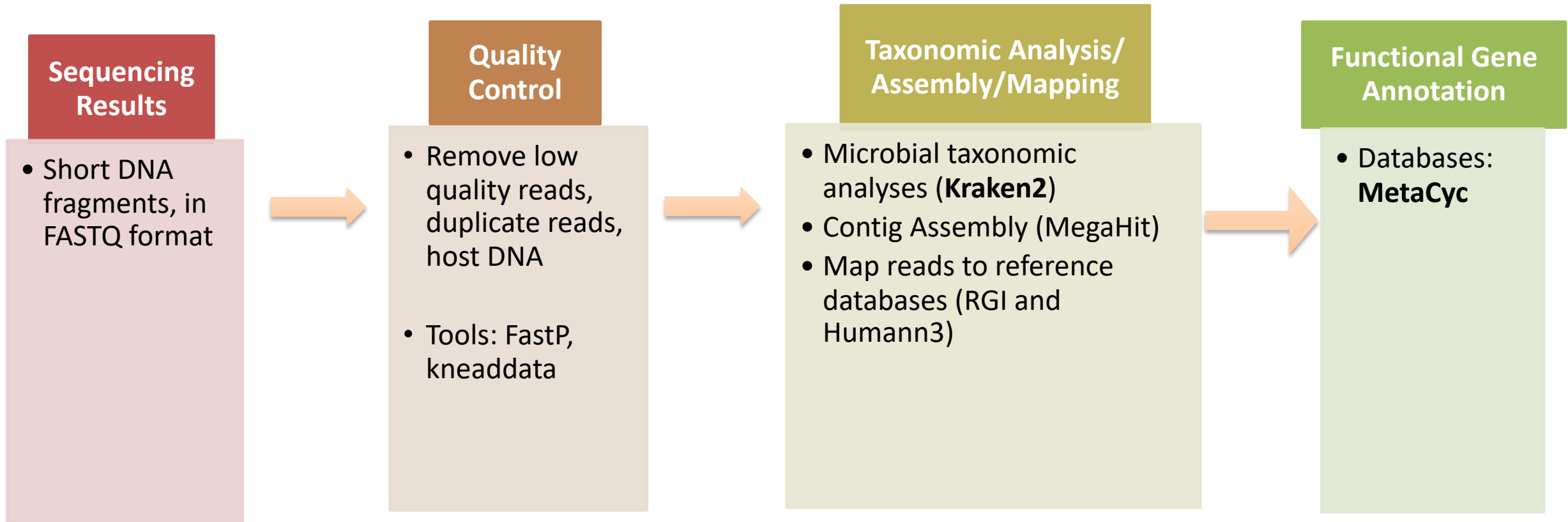
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Cycle 3	WW	WW	WW	WW	WW	FD	FD
Cycle 4	WW	WW	FD	FD	WW	FD	FD

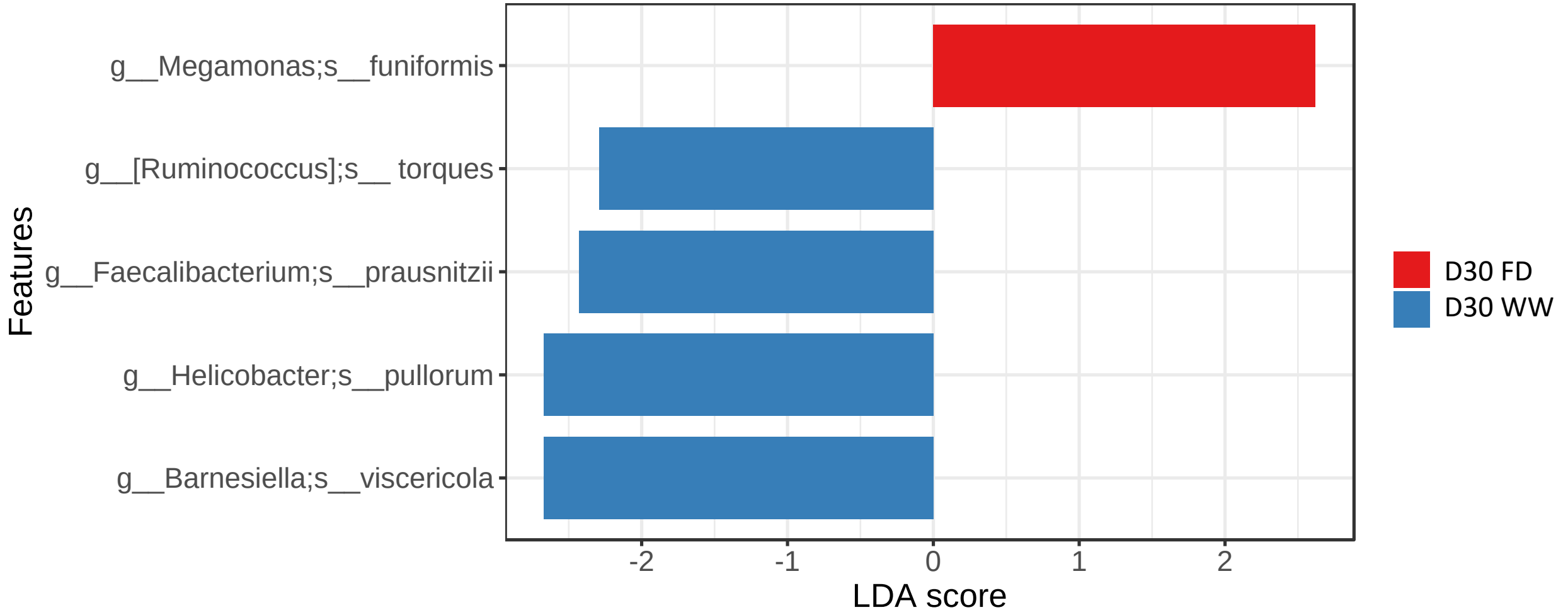
- 7 Water-Wash flocks vs. 7 Full Disinfection flocks

Shotgun Metagenomics Sequencing



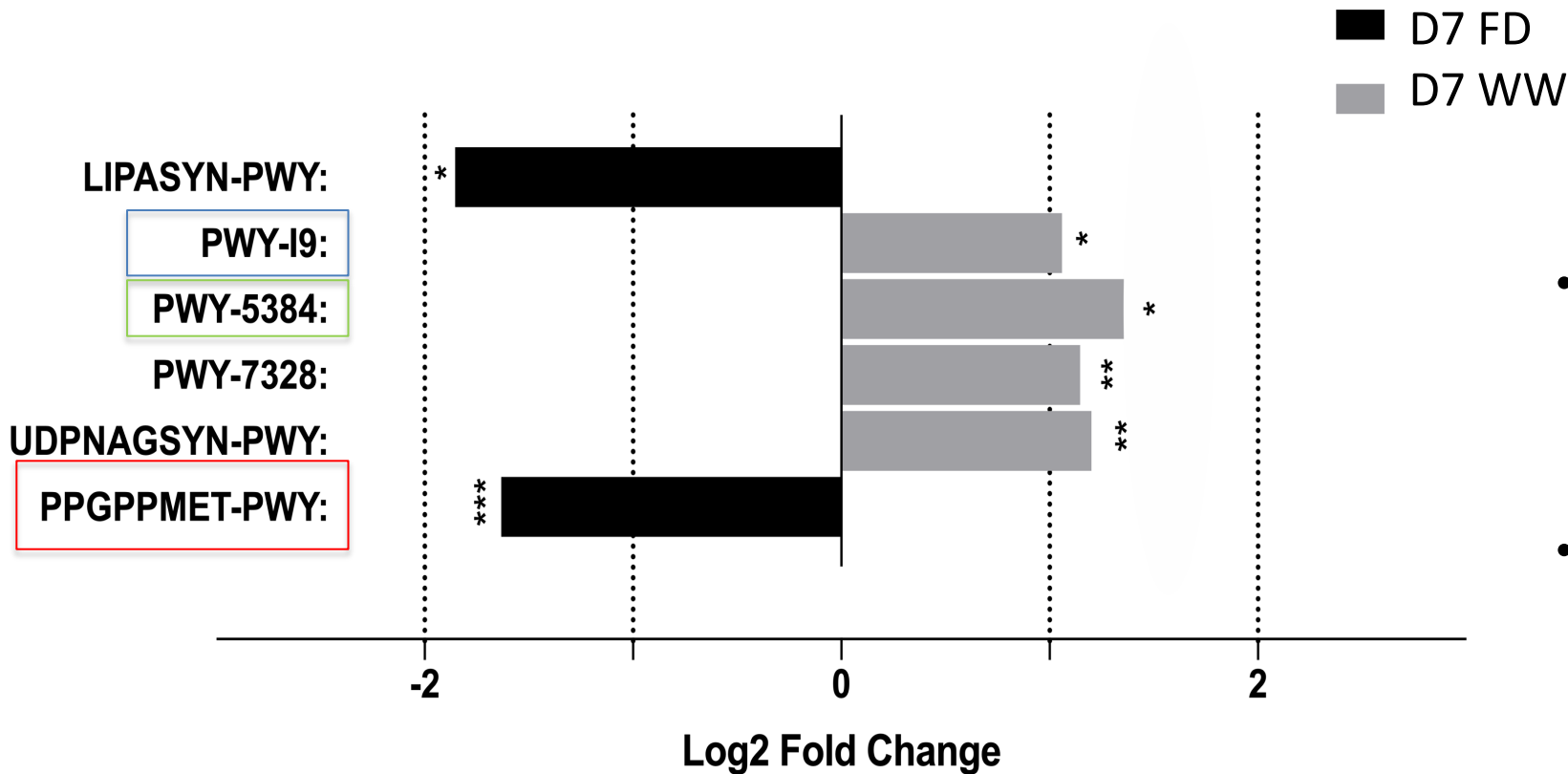


Results: D30 Cecal Microbiota



LEfSe results: FDR $P < 0.05$, LDA > 2

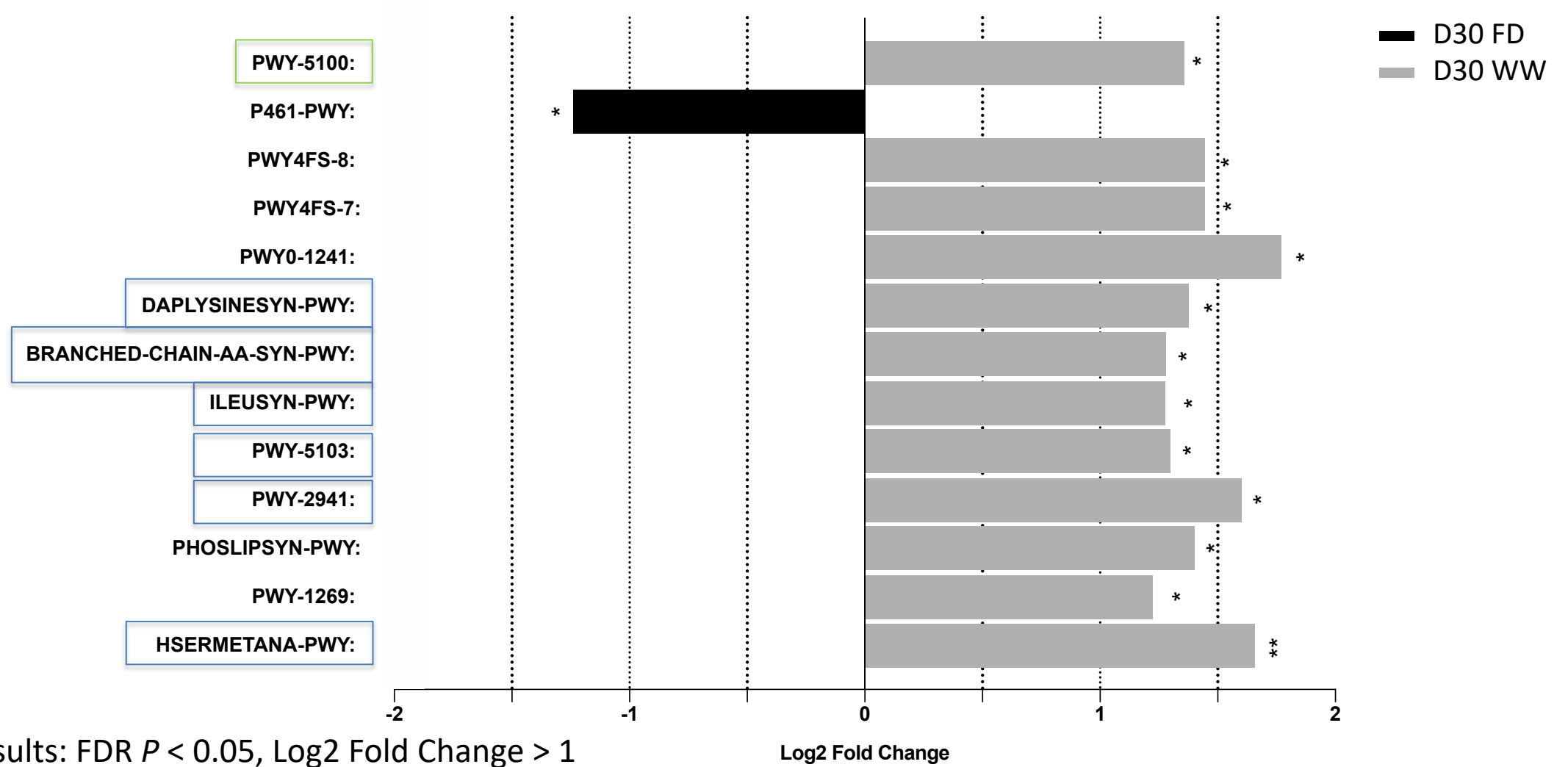
Results: D7 Microbial Functionality



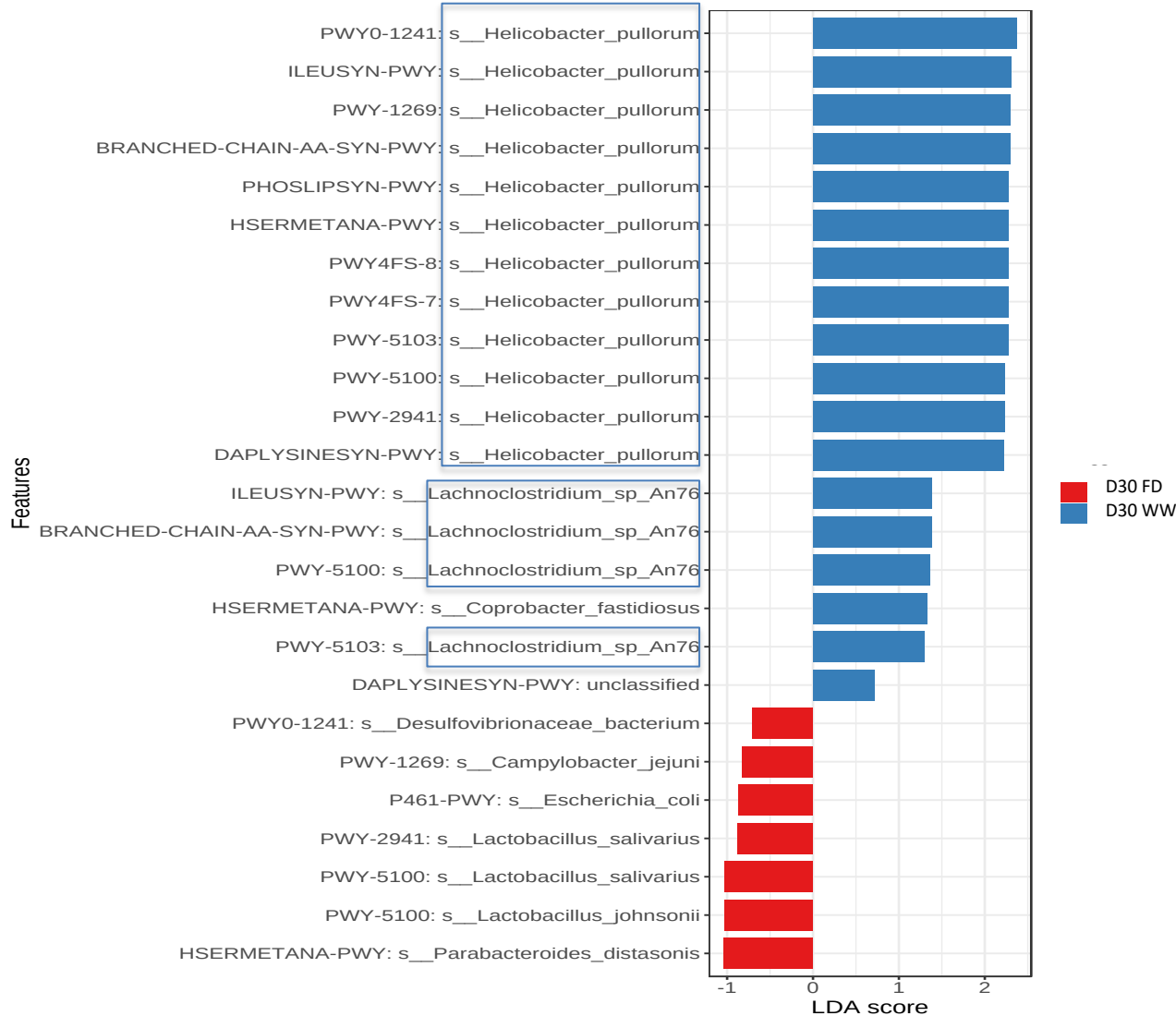
- Enriched pathways linked to polysaccharide degradation and amino acid synthesis
- Enriched pathway linked to stress responses in the FD group

DESeq2 results: FDR $P < 0.05$, Log2 Fold Change > 1

Results: D30 Microbial Functionality



Results: Species Contribution (D30)



- The differentially abundant pathway was contributed by 62 different bacteria species
- The increased abundance of *H. pullorum* in the ceca of the D30 WW chickens greatly contributed to all enrich pathways in the WW group
- *Helicobacter pullorum* was considered as an opportunistic pathogen (Javed et al, 2017), but **it was NOT directly** associated with human (Mulatu et al., 2014) or poultry (Ceelen et al., 2005) diseases

- At the end of the cycle, WW and FD had comparable flock mean body weight and mortality rate
- The WW group had decreased cecal *C. jejuni* abundance at D30
- Barn cleaning methods had a subtle but significant impact on the chicken gut microbial community
- The WW group had increased SCFA concentrations in the ceca, which was negatively associated with *C. jejuni* abundance
- The WW-derived microbial functionality had enriched amino acid biosynthesis and SCFA biosynthesis capacities, where *H. pullorum* may be a key member in the altered microbial functionality

- Compare barn sanitation practices in a more controlled environment/facility.
- Perform transcriptomic and proteomic analyses
- Seed *H. pullorum* and define communities to germ-free chickens.

Supervisor: Dr. Ben Willing
Dr. Doug Korver



Co-lab partners: Dr. Lynn McMullen
Dr. Tom Inglis



The Willing Lab
The Korver Lab
My Colleagues: The McMullen Lab
Poultry Health Services



Questions?

