



Consortium Program 4

Program leader: Professor Dana Stanley

Precision Gut Colonisation

Professor Dana Stanley

Maria Whitton

Fatemeh Sharifi

Yadav Bajagai

Sung J. Yu

Early colonisation

- **Gut microbiome** influences many aspects of health
- Gut colonisation can **establish the gut microbiota** that will dominate the animal's life
- First two days of life are critical - intensive colonisation
- The first feed is the most important meal
- Probiotics are live microbes that benefit gut health and can outgrow pathogens
- Prebiotics are indigestible fibres; act as food for beneficial gut **microorganisms**, glycans have major role
- This colonisation is the window where a **small intervention** can **prevent pathogens** from colonising and improve health **permanently**



Industry is catching up...

- On-farm hatching
- HatchCare systems from HatcherTech
- Early-life essentials that are proven to support chick health, growth and uniformity.



Glycans

- Glycan prebiotics are complex carbohydrate molecules that serve as food for beneficial gut bacteria, promoting a healthy microbiome and immune function
- Examples include naturally occurring prebiotics like fructo-oligosaccharides (FOS) and galacto-oligosaccharides (GOS), as well as mucin glycans
- **Natural glycans** (FOS, GOS, plant glycans, mucin glycans)
- **Synthetic glycans** and precision glycans (engineered for specific effect on microbiome)
- Synthetic mucins mimic the function and structure of natural mucins; used for **cancer, antiviral, vaccines (include pathogen glycol-structures), drug delivery**

Precision glycans alter gut colonisation

- Artificially synthesised prebiotics with **specific 3D structure** to trap pathogens
- PG's have **action on the mucin layer**
- **Mucin is in direct contact with intestinal epithelial cells**
- Evidence suggests they have a major role in what species can colonise the mucin layer
- **Reducing pathogen access to epithelial cells.**

Hypothesis

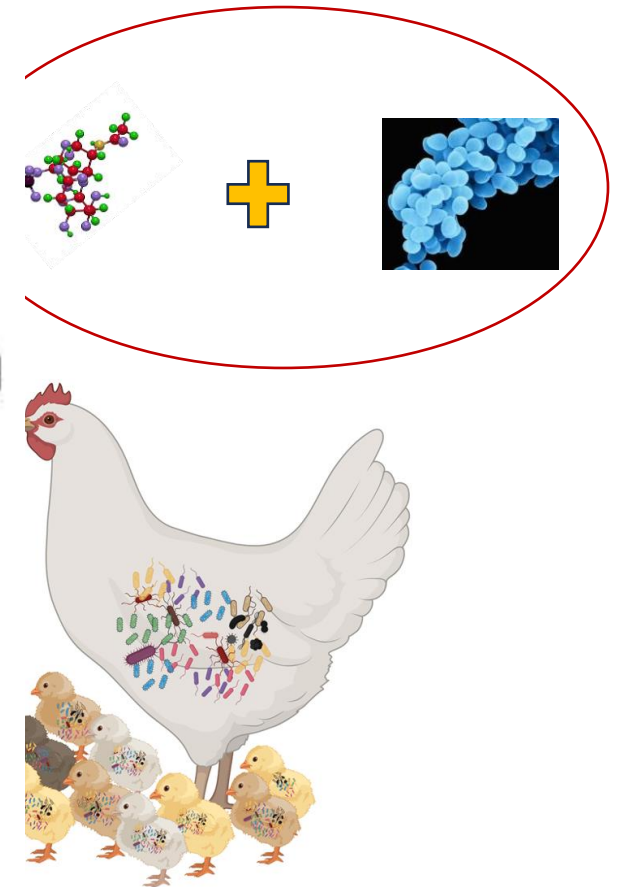
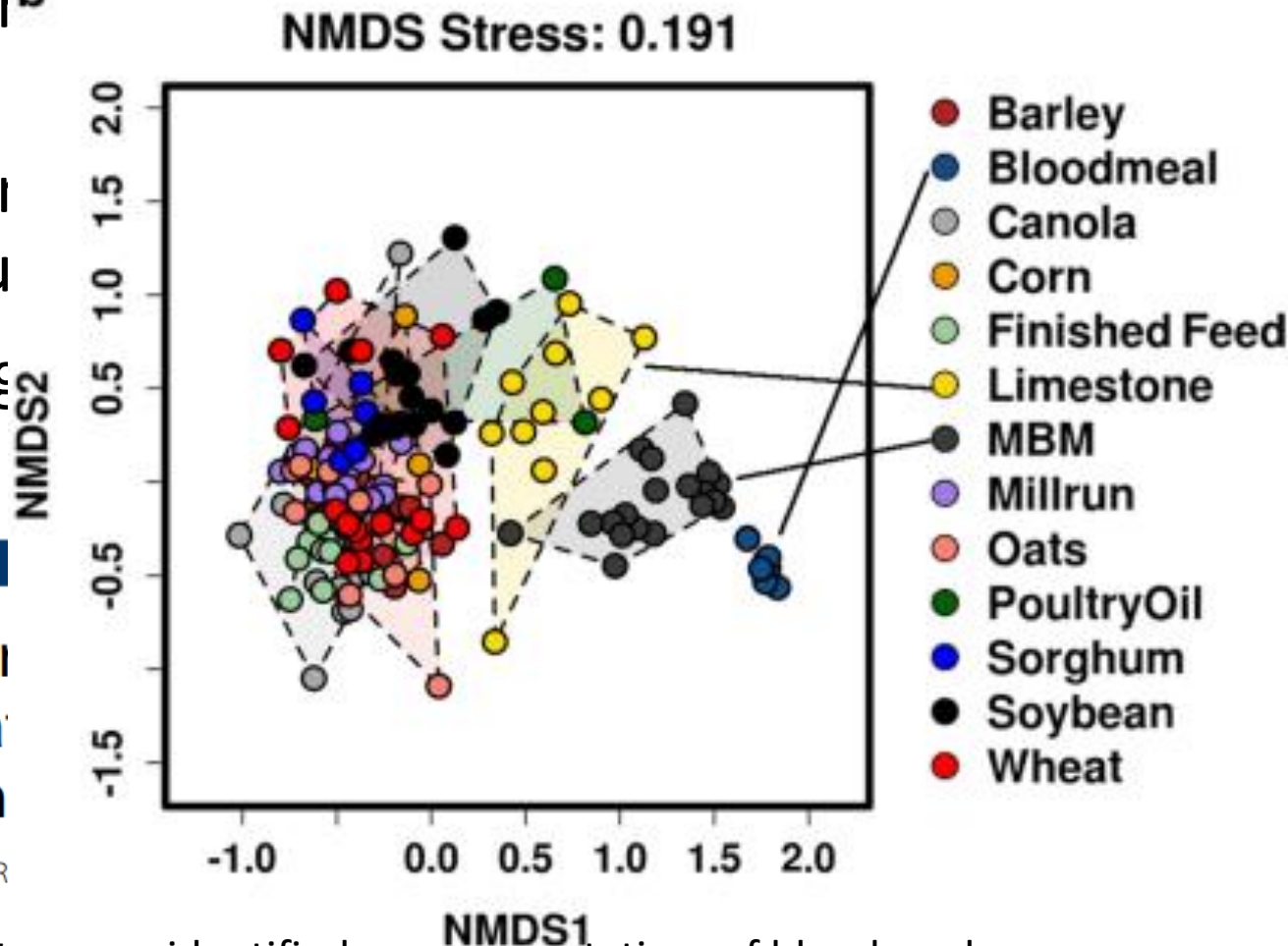
- Early administration of a PG and compatible probiotic will have major establishment
- First feed like it reshape the gut
- Prevent pathogen preferred feed

ORIGINAL ARTICLE

Poultry feeds can shape gut microbiota communities that

Sarah Haberecht^{1†}, Yadav S. Bajagai^{2†}, R

- *Clostridium* and *Streptococcus* identified as representatives of bloodmeal



Trial 1

Objective:

Evaluate whether precision glycan (PG) supplementation can influence early gut microbial colonisation and optimise PG dose.

- **Four** treatment groups.
- Precision glycan (**PG**) was supplemented **only in the first 48 hours** to improve gut colonisation.
- Normal diet for the rest of the trial
- At week 4 the birds were challenged with DEX to introduce leaky gut.
- At day 28, the birds were euthanised, and samples were collected from ileum content, ileum mucosa, and caecum content for microbiota.
- Samples for histology and RNAseq were collected from the ileum and liver



Trial 1:

- Altered community structure in Ileum and IM, no change in caecum
- Swabs collected weekly for microbial maturation timeline

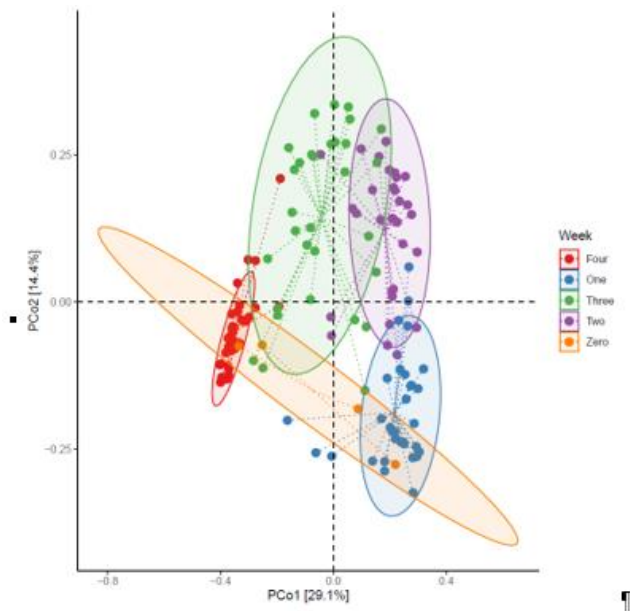
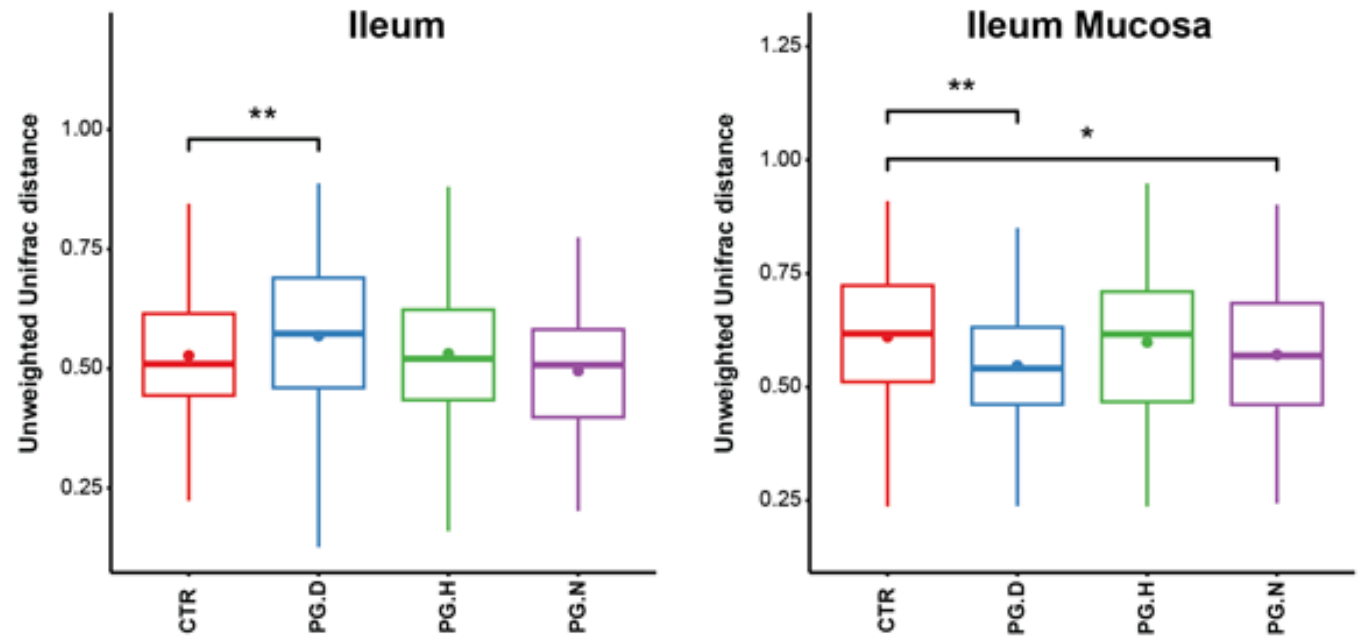


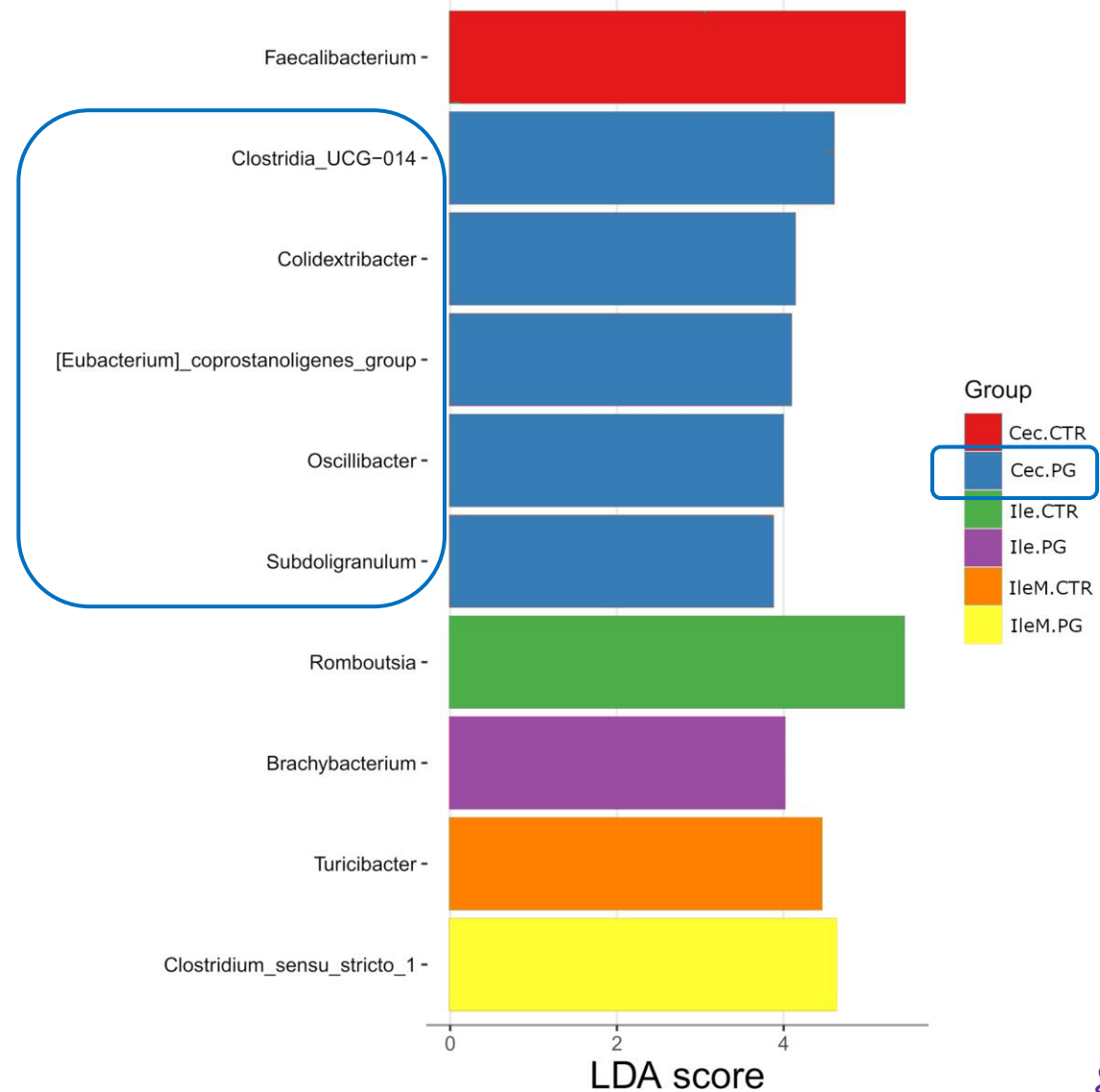
Figure 2: Succession of litter microbiota from week 0 to week 4 measured with Unweighted UniFrac distance. Week 4 was a week of leaky gut/DEX challenge.



LEfSe

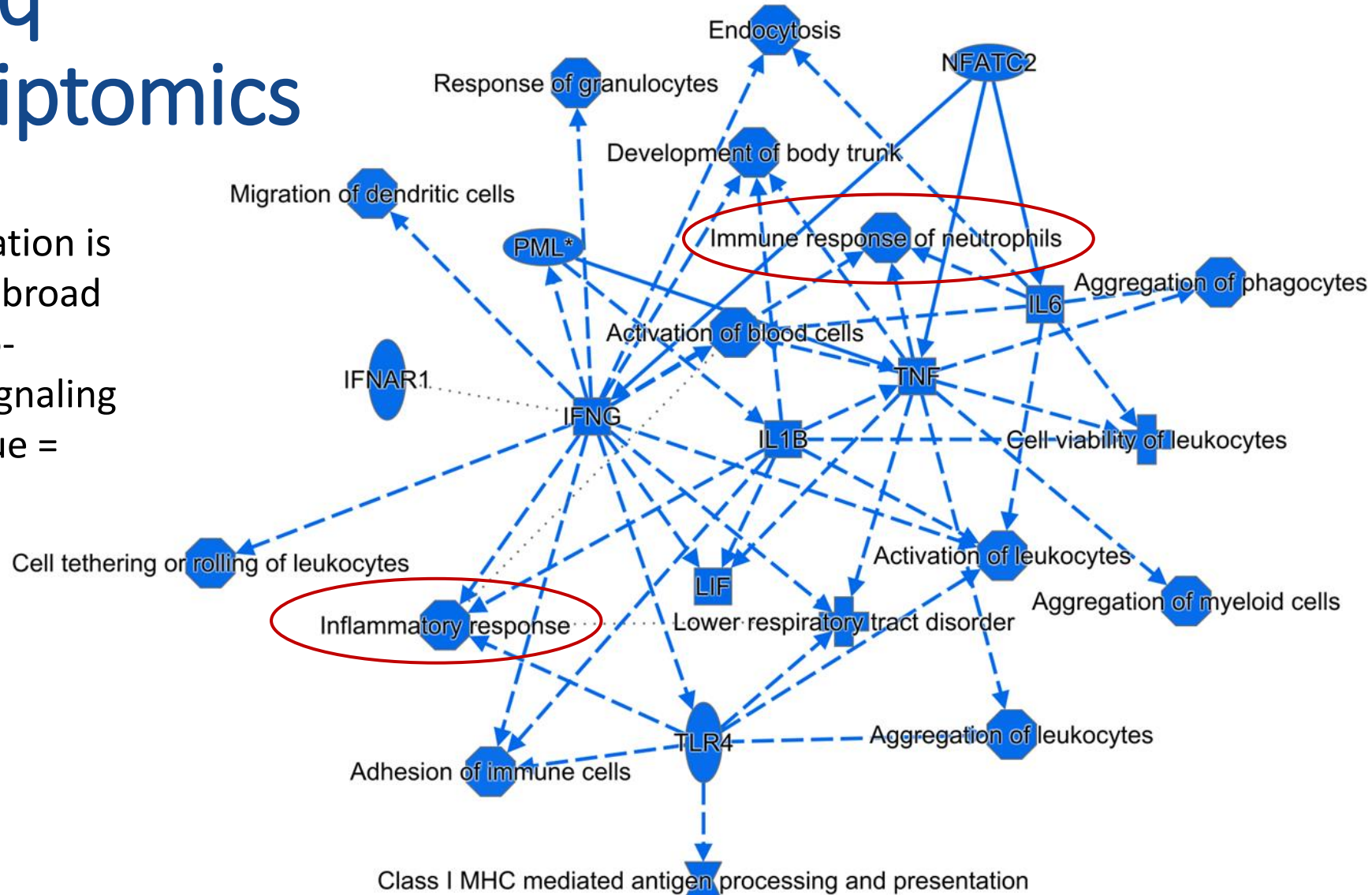
Butyrate producers maintain gut health as a major source of energy for host cells.

Producers of SCFAs (especially Butyric and Propionic)
Positively associated with barrier function, mucus secretion and repair of the intestinal barrier.



RNA seq transcriptomics

PG supplementation is associated with broad inhibition of pro-inflammatory signaling in the ileum (blue = reduced)

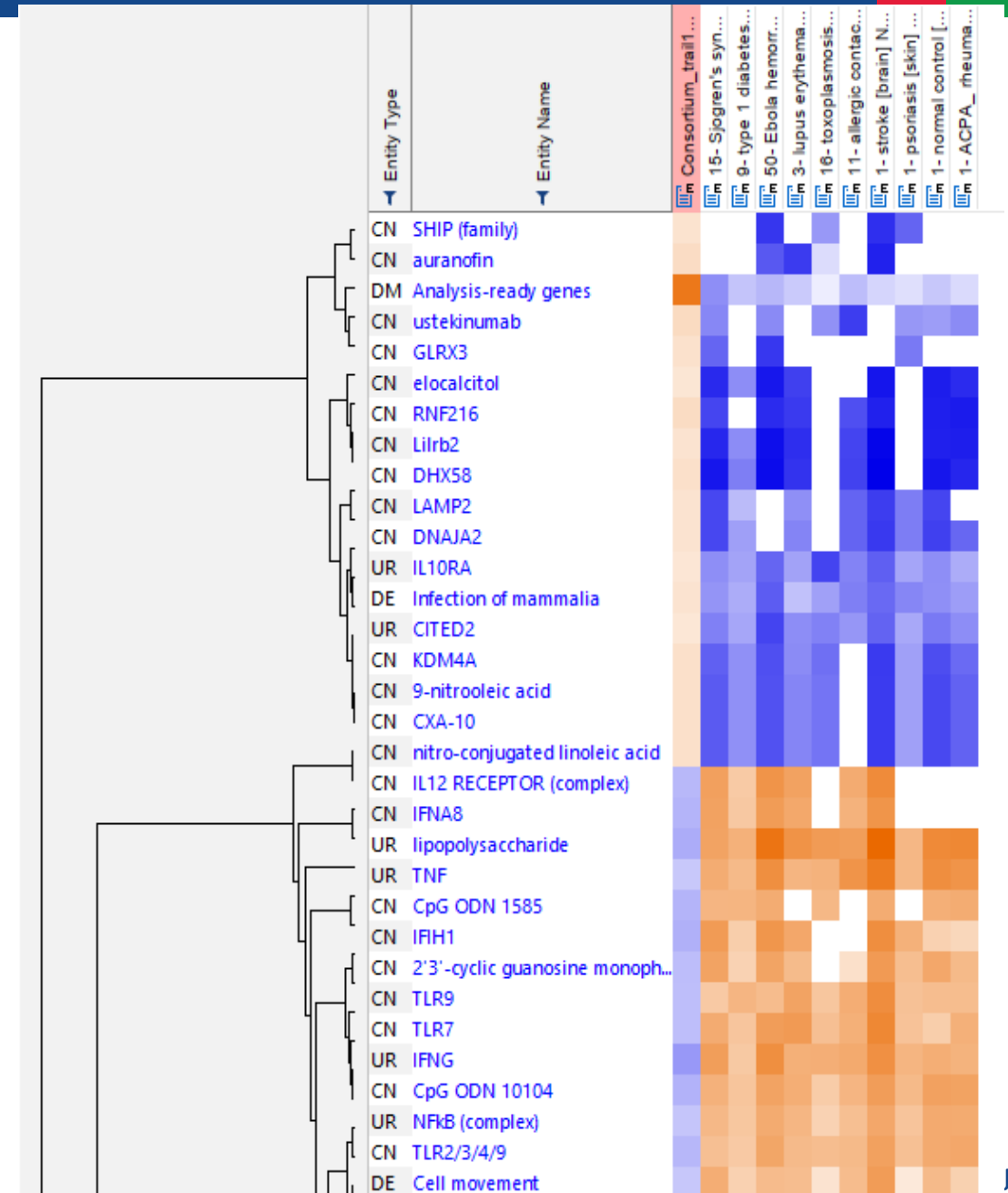


Upstream analysis

- It reported that the effects of administering PG during the first 48 hours only, result in changes notable after the DEX challenge at 4 weeks of age that are significantly **similar to the transcriptomic effects of curcumin**
- **and significantly opposite (capable of undoing the effects of) E. coli LPS on ileum**
- Strong inhibition of IFNG, TNF, NF-κB

Upstream analysis

- Opposite to gene expression effects of
 - Sjogren's syndrome,
 - Type 1 diabetes,
 - Ebola infection,
 - Lupus,
 - Toxoplasmosis of the spleen,
 - Allergic contact dermatitis (ACD),
 - Stroke,
 - Psoriasis,
 - LPS and
 - Rheumatoid arthritis.
- Positive effects were not linked to cecal SCFA post DEX



Trial 1 conclusions

Benefits of Precision Glycan (PG) Supplementation

- **Changed the composition of gut microbiota – altered colonisation**
- **Modulates immune response** by reducing overactivation of inflammatory pathways (e.g., TLRs, cytokines).
- **Protects gut integrity**, lowering risk of leaky gut and tissue damage.
- **Enhances bird resilience** under stress (e.g., dexamethasone challenge).
- More trials needed for performance

Trial 2

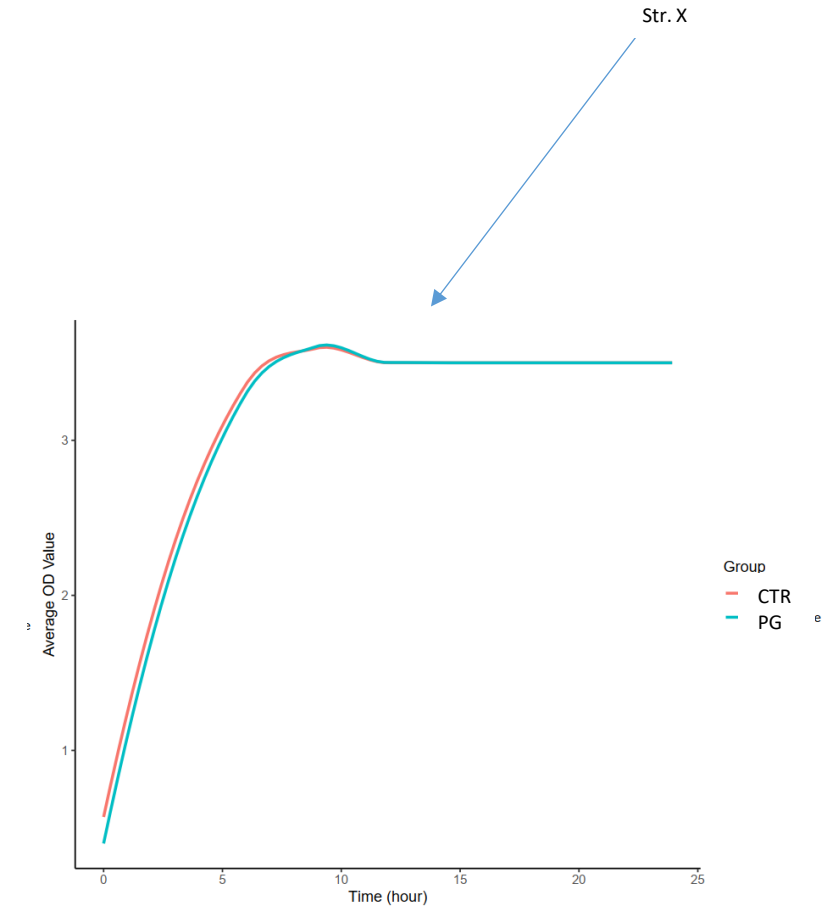
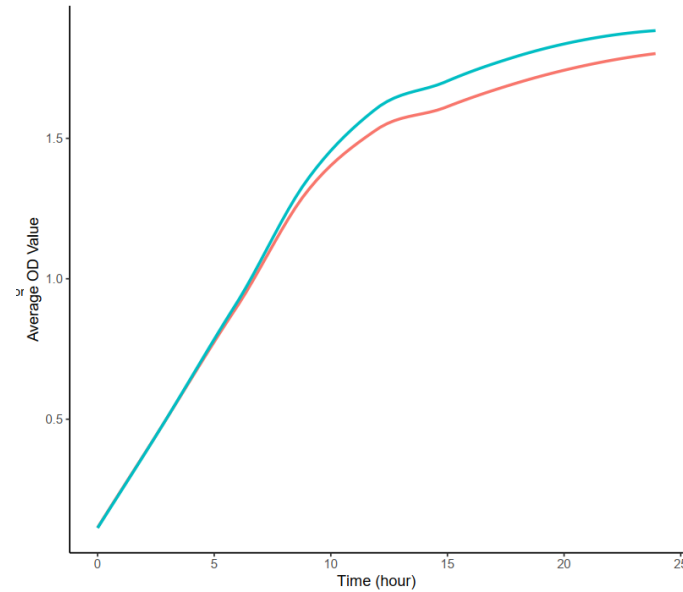
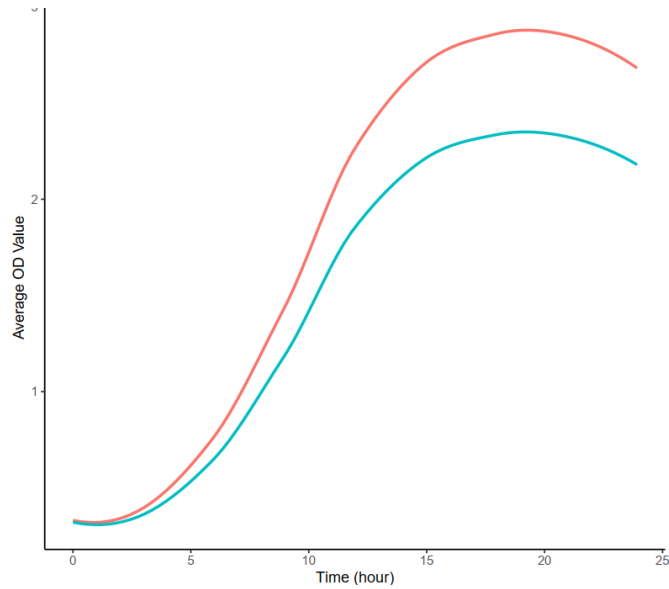
Objective: Determine the impact of the PG-compatible probiotic strain

- 3 Treatments
- **PS-fermented feed** for the **first 48 hours**.
(bacteria grew in starter crumble for 24 hr, then dried)
- Basal diet until the end.
- **Dex challenge** at week 4
- Samples taken for 16S microbiota, histology, RNA-seq, SCFAs taken at day 28.

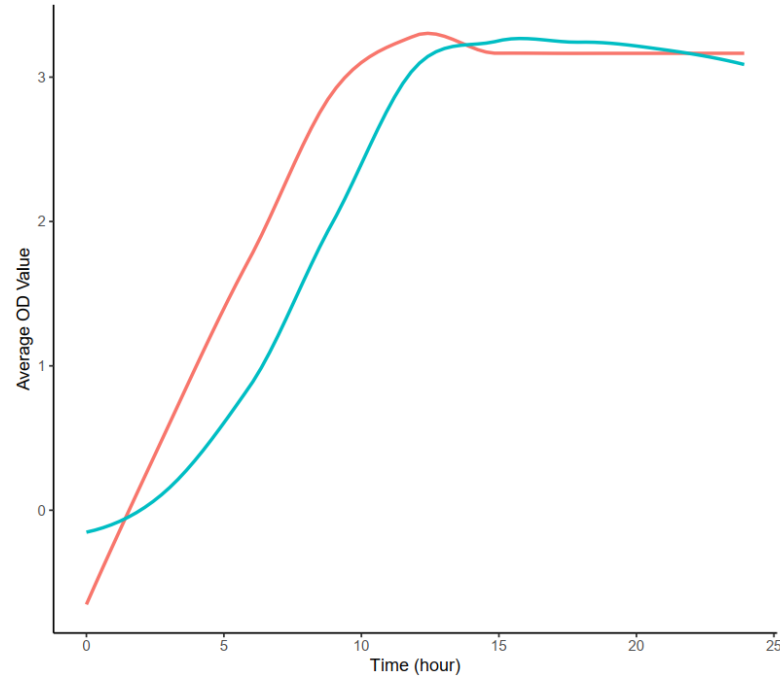
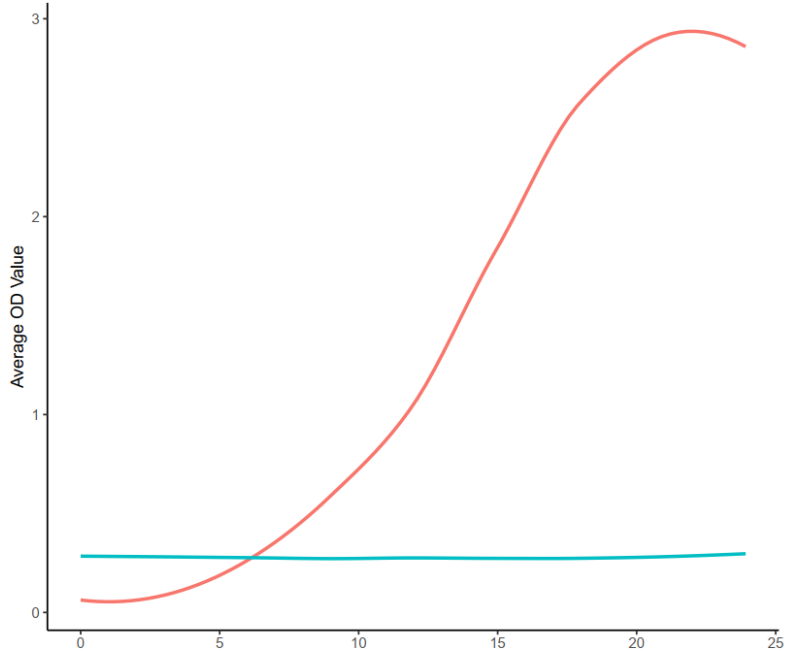


In vitro work:

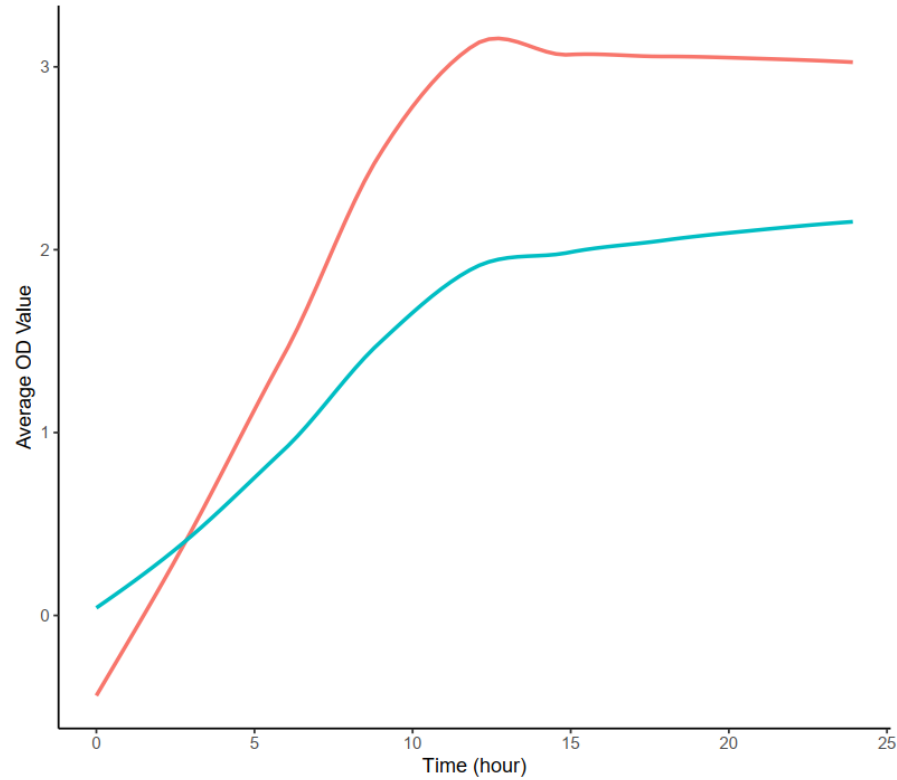
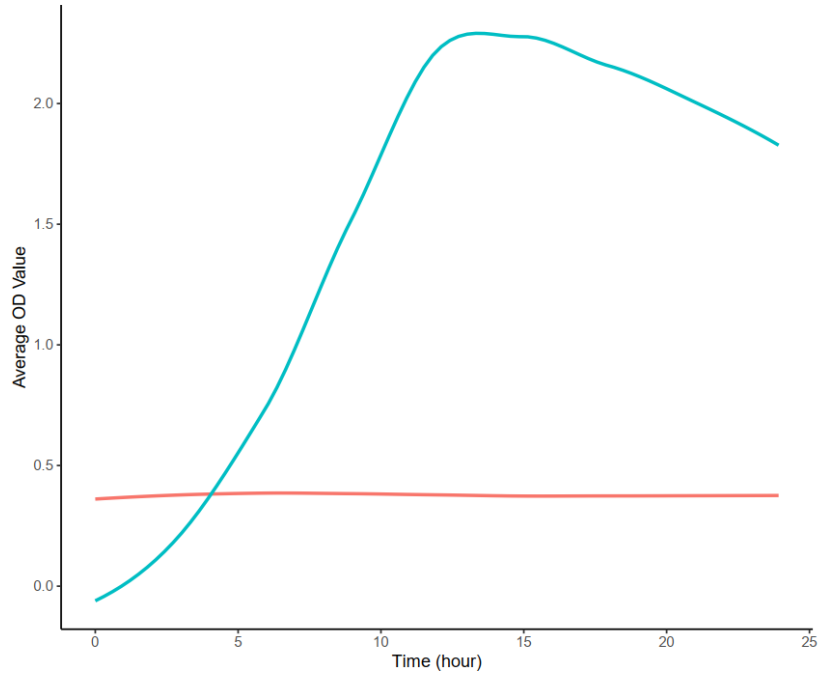
- Extensive in-vitro work is still ongoing
- PG in combination with 200 probiotic strains
- Test 1: Strains grown together with PG



Test 2: Are they acid tolerant?



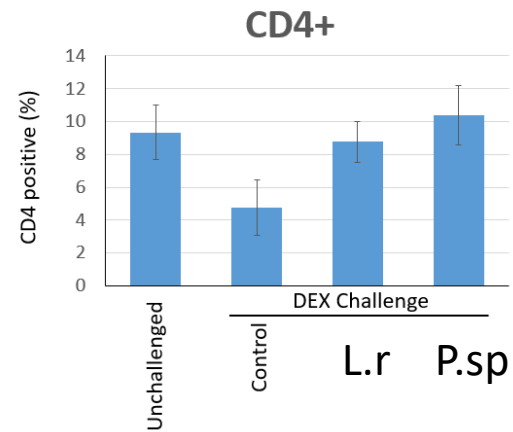
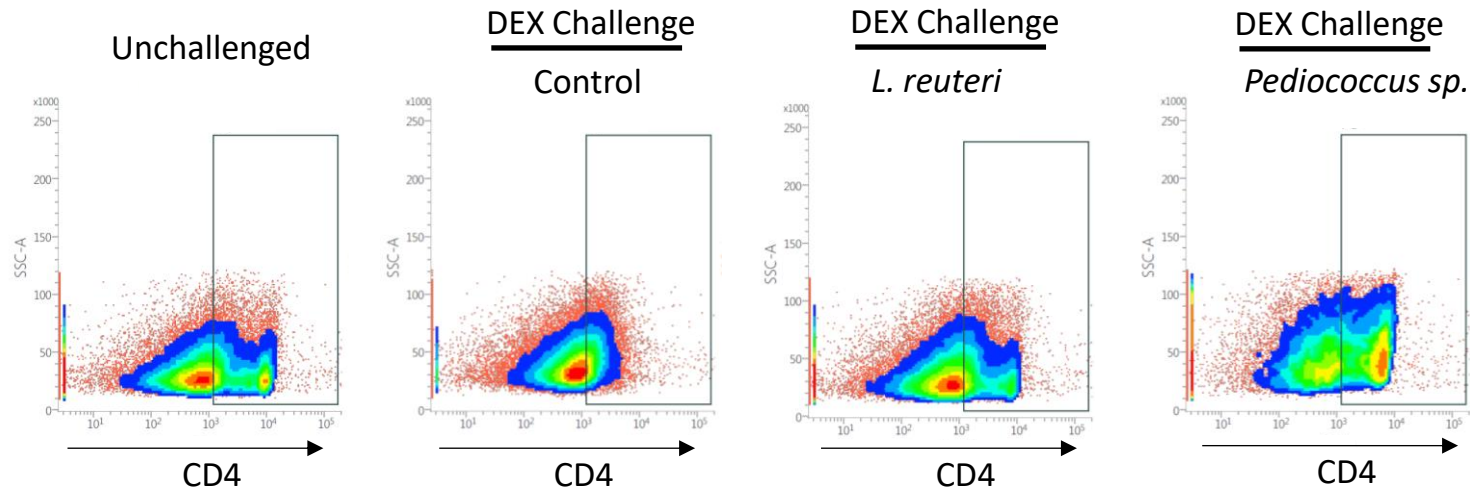
Test 3 – Bile tolerance



- Selected *L. reuteri* or *Pediococcus sp*



The T helper cells (Th cells), also known as CD4+ cells or CD4-positive cells



Flow cytometry on:

- Caecal tonsils
- Spleen
- Thymus
- Bursa
- Blood

Pediococcus sp. restores Th cells

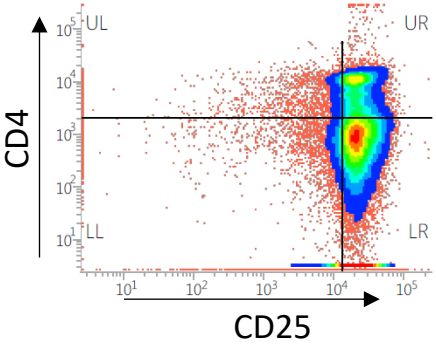
- DEX reduces CD4 positive cells on cells in the Bursa
- The T helper cells (Th cells), also known as CD4+ cells or CD4-positive cells, are a type of T cell that play an important role in the adaptive immune system.
- Administration of *L. reuteri* or *Pediococcus sp.* rescues the expression of CD4 back to normal (unchallenged) levels
- Similar results were observed in cecal tonsil

The Australian

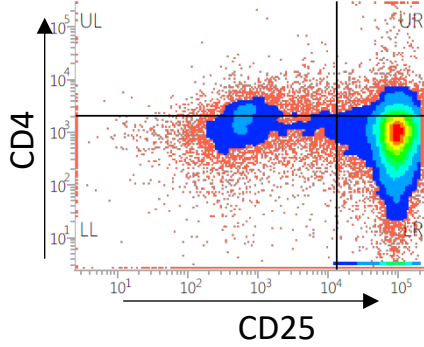


Pediococcus sp. restores Treg cells

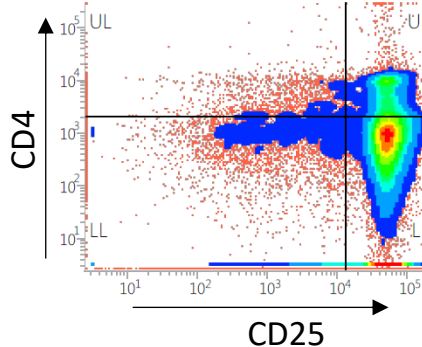
Unchallenged



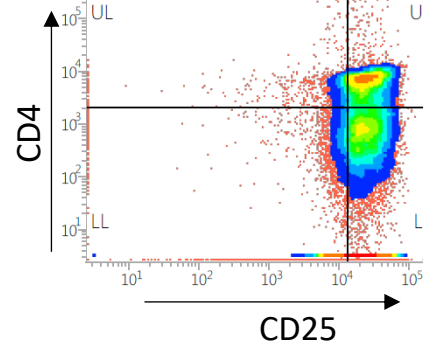
DEX Challenge
Control



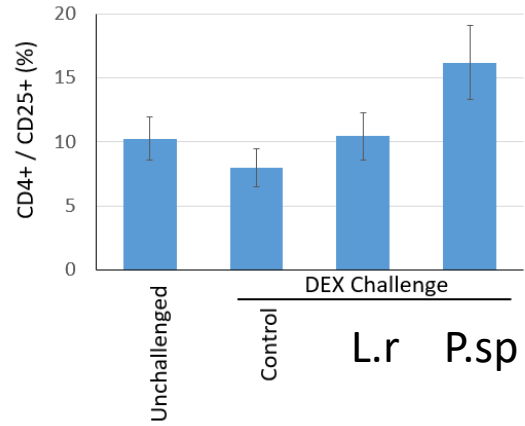
DEX Challenge
L. reuteri



DEX Challenge
Pediococcus sp.



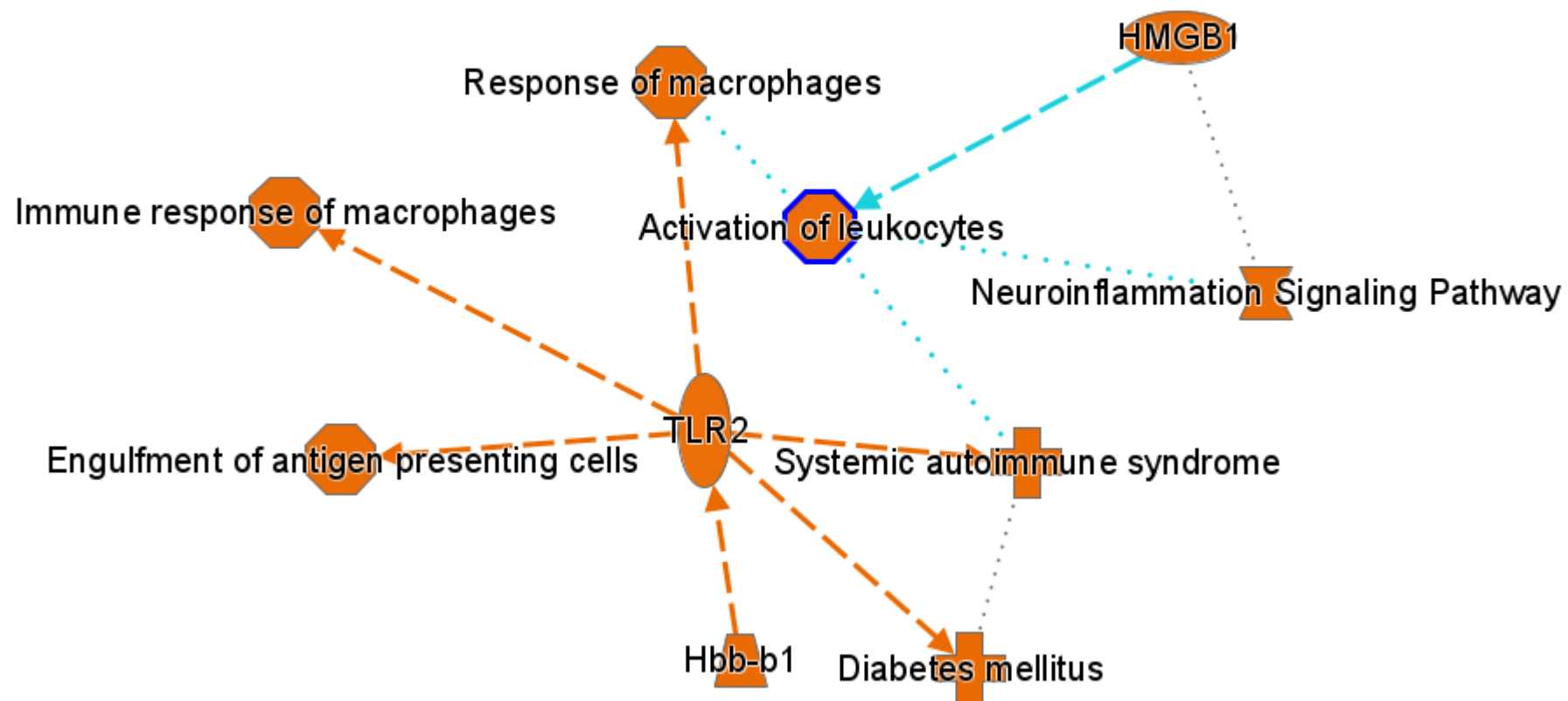
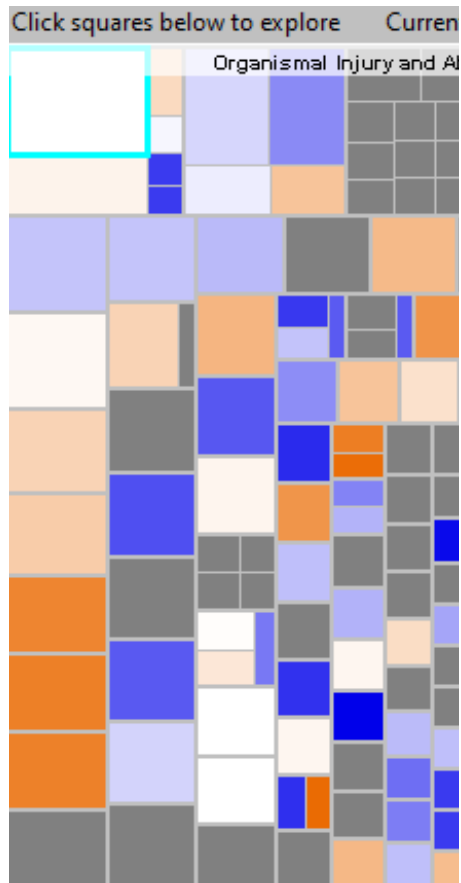
Dual CD4+ / CD25+



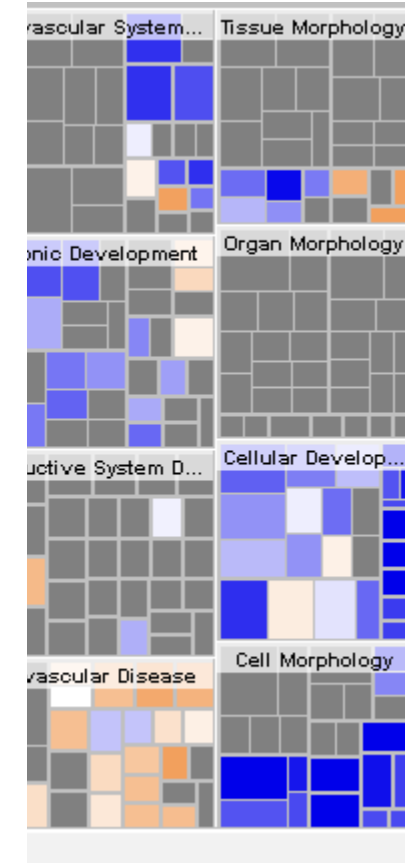
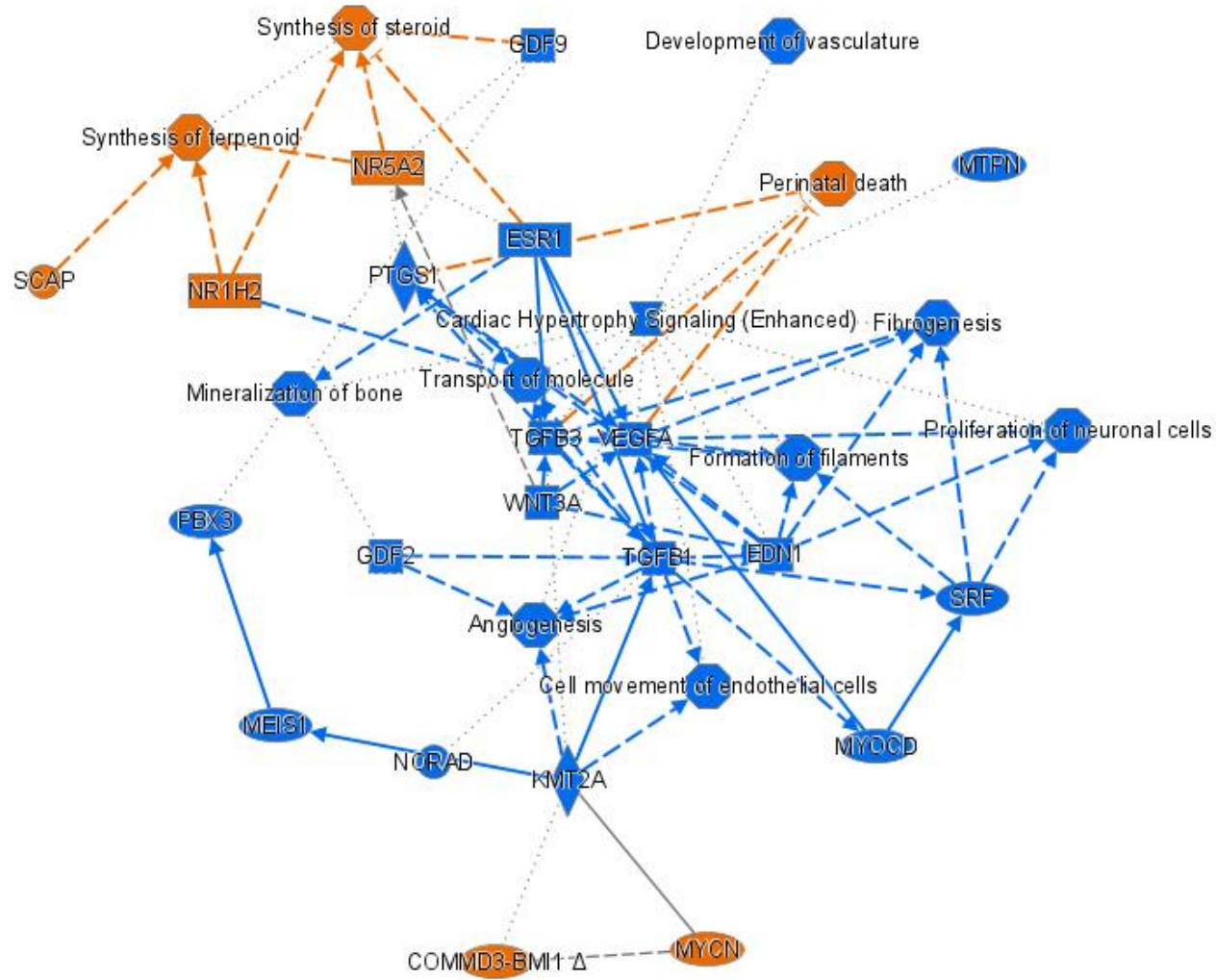
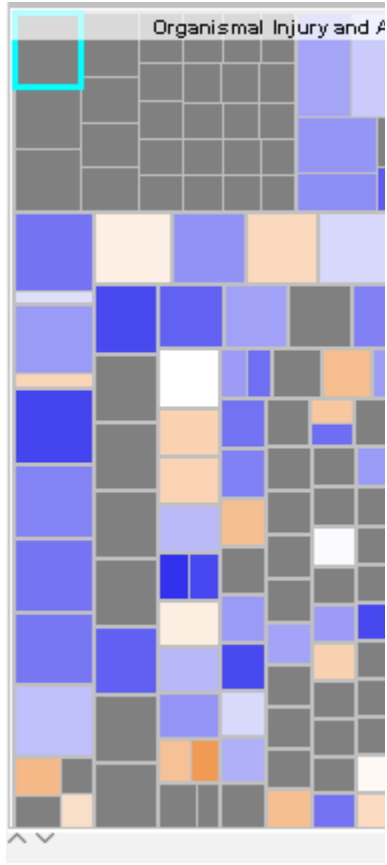
Pediococcus sp. restores Treg cells

- DEX reduces CD4 / CD25 positive cells in the Bursa
- CD4 / CD25 positive cells are T-regs
- CD4+CD25+ T regulatory (Treg) cells control **immunologic tolerance** to self-antigens and play a role in suppressing antitumor immune responses, **they tame immune system overreaction**
- Administration of *L. reuteri* or *Pediococcus sp.* rescued the number of CD4 / CD25 positive cells back to normal (unchallenged) levels or beyond
- We also performed flow cytometry on Cecal tonsil and bloods (not shown)

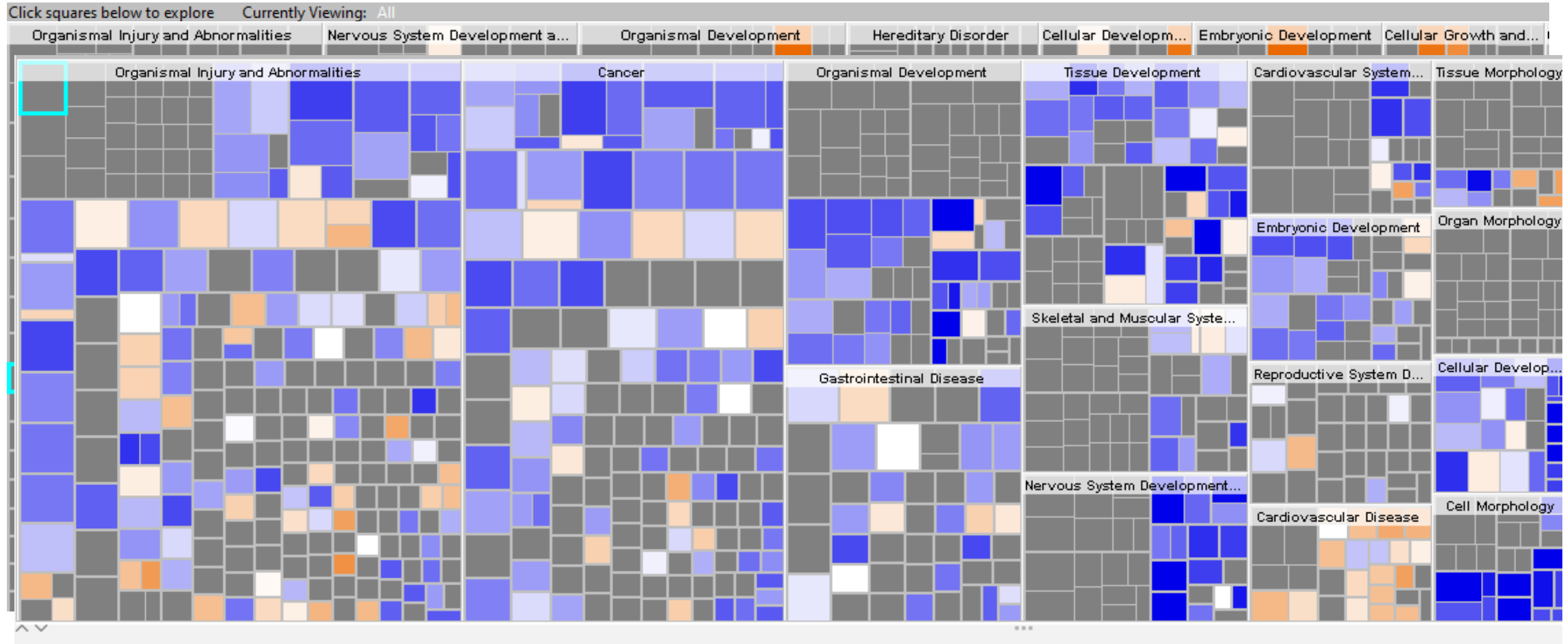
DEX challenge



Pedic



Summary – Disease heat map



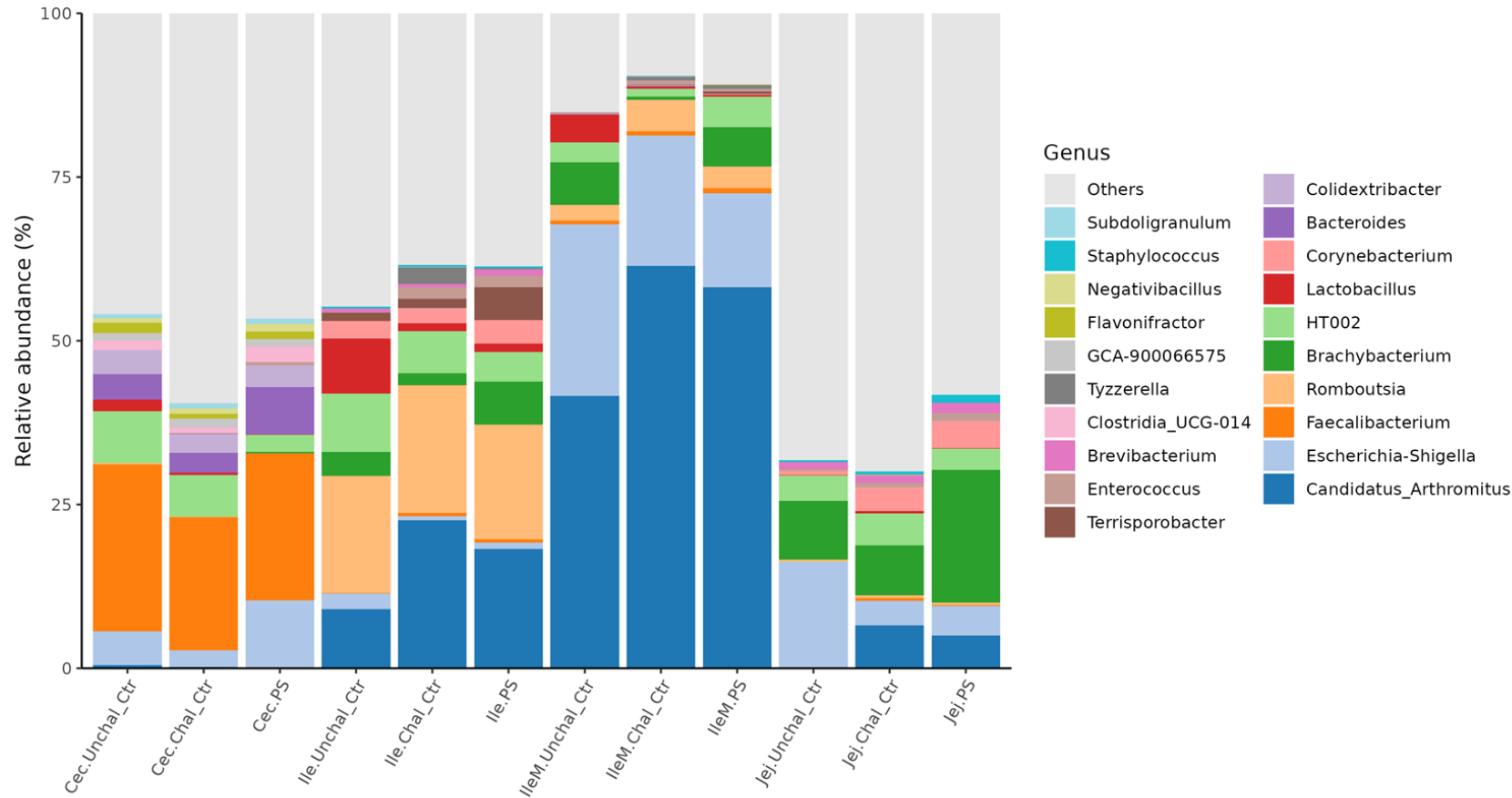
Trial 2

Objective: Determine the impact of the PG-compatible probiotic strain *Pediococcus sp.* (PS).

- 3 Treatments
- PS-fermented feed for the **first 48 hours**. (bacteria grew in starter crumble for 24 hr, then dried)
- Basal diet until the end.
- **Dex challenge** at week 4
- Samples taken for 16S microbiota, histology, RNA-seq, SCFAs taken at day 28.

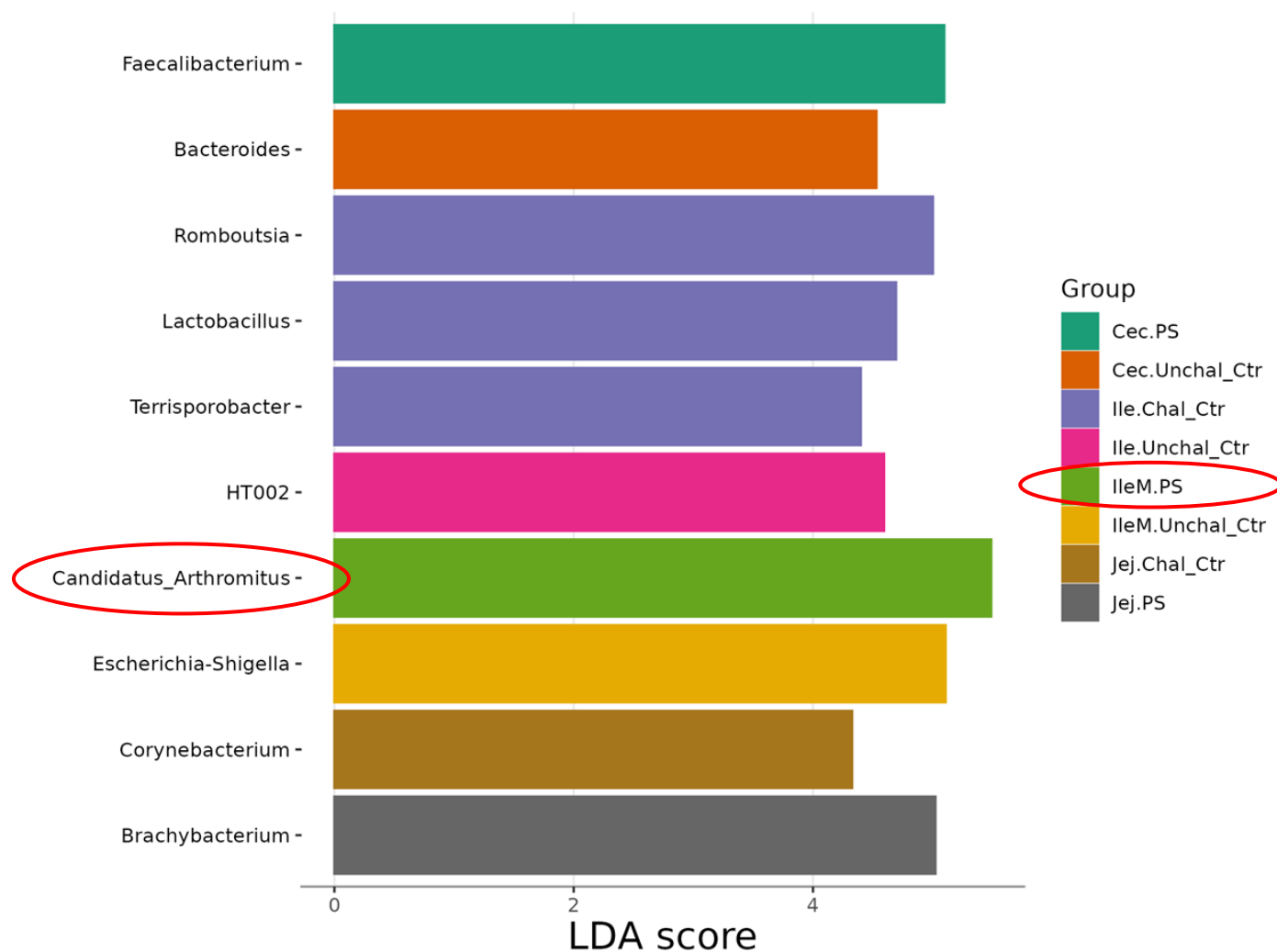


Microbiota composition



Community structure changes not only by organ, but also changes between treatments

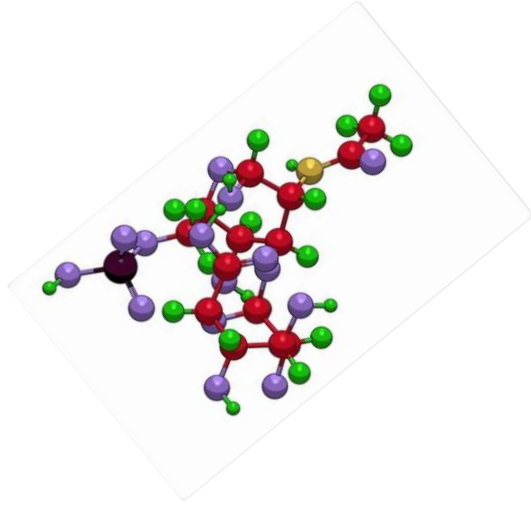
LEfSe- Differential genera



This genus plays a role in immune system maturation when interacting with the host.

CA attaches to the intestinal epithelium without causing inflammation and triggers an immune response that blocks pathogens from colonising epithelial receptors.

Are PG and the chosen probiotic *Pediococcus* compatible?



PG focuses on **reducing inflammation** and preserving energy



Probiotic focuses on **restoring immune capacity**

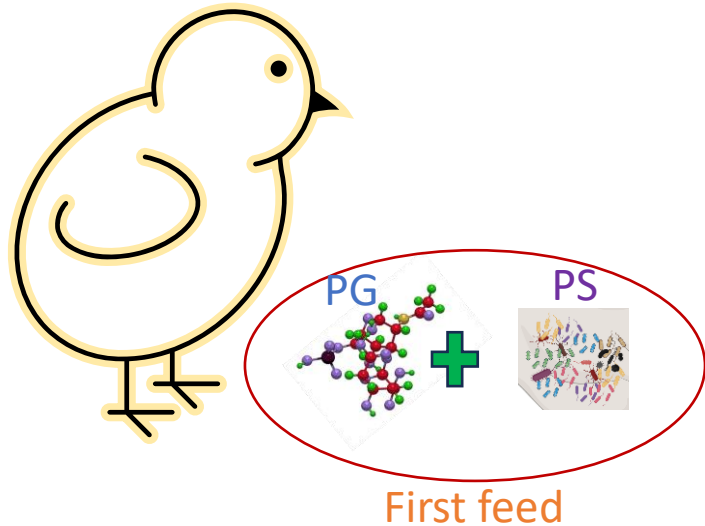


This will be useful in commercial poultry production under stress, where both **immune efficiency and resilience** are needed



Trial 3

Objective: Assess whether combining PG with its compatible probiotic alters gut colonisation.



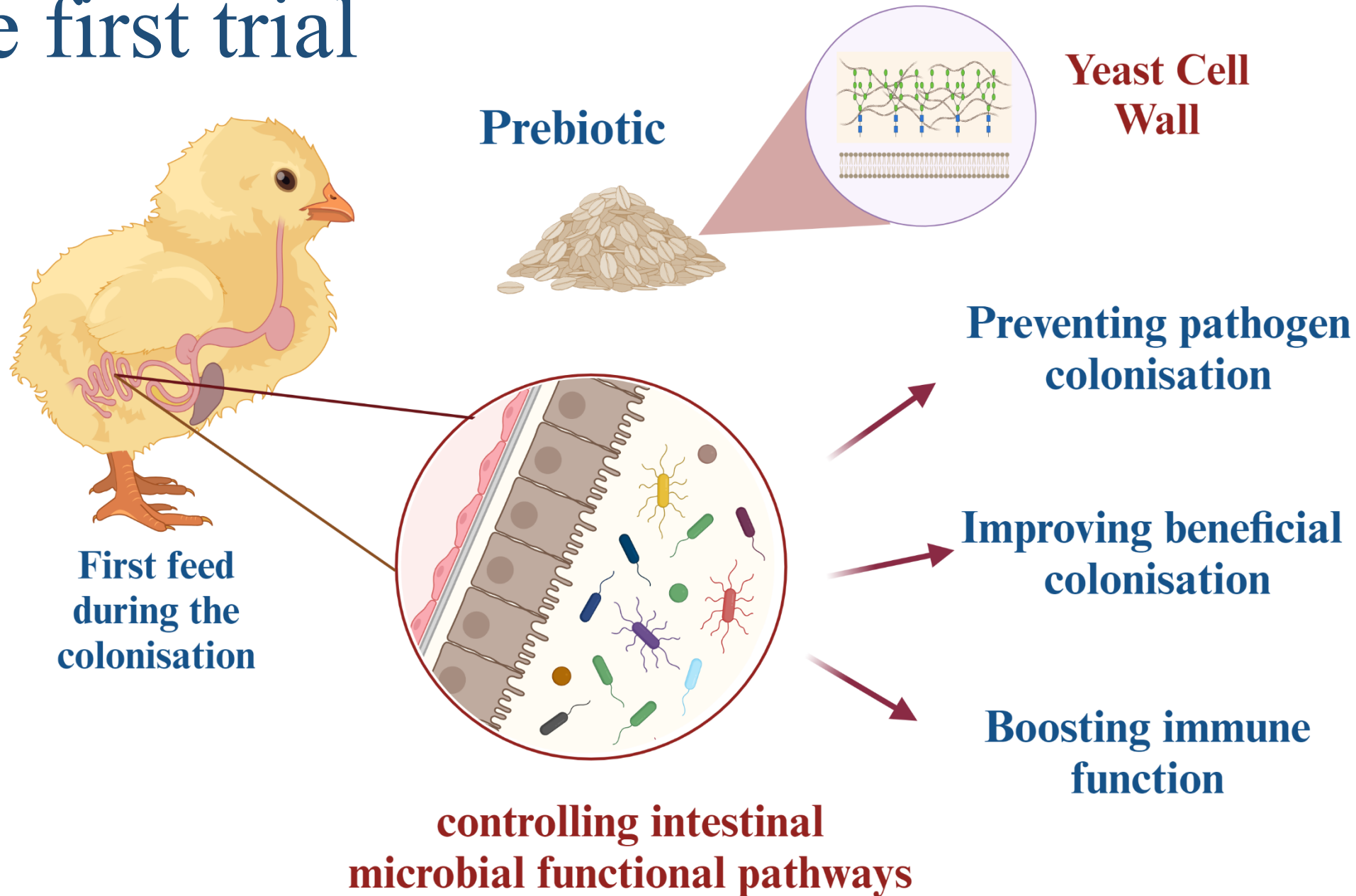
- 3 treatments. 8 replicates per treatment.
- **Treatment for the first 48 hours**
- Basal diet until the end
- **Dex challenge** at week 4
- Day 28 samples taken for 16S microbiota, histology, RNA-seq, SCFAs.
- Results are coming soon.

Fatemeh Sharifi joined a year later...

- Yeast cell wall glycans are complex polysaccharides, primarily composed of **β -glucans** and **mannoproteins**
- Can interact with and modulate the gut microbiota by promoting beneficial bacteria
- Reducing pathogens
- Alter metabolome (SCFA)
- Immuno-modulation
- Supplements for human and animal health



The first trial



Methodology

- ❖ **Animal trials**
- ❖ **Microbiota sequencing profile**
- ❖ **Metagenomic sequencing for functional analysis**
- ❖ **RNA sequencing**
- ❖ **Histology and immunohistochemistry**



Experimental Design

❖ Location & Facilities

- Animal trial conducted at CQIRP, Rockhampton.
- Fertilised Ross 308 eggs incubated using Brinsea Ova Easy 380 Advance EX Series II. Temperature: 37°C, Humidity: 55–57%, Duration: 21 days until hatch

❖ Post-Hatch Allocation

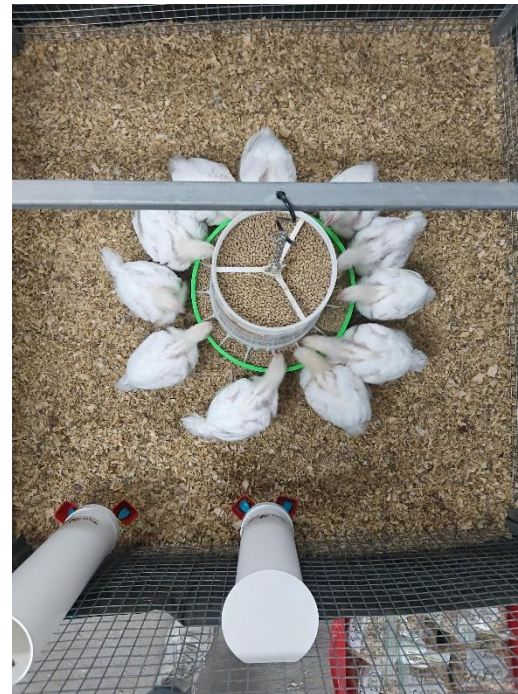
- Chicks allocated randomly to 1.2 × 1.2 m pens.
- Total chicks: 160 → 16 pens → 10 chicks/pen.

❖ Treatments (Trial 1: At-Hatch YCW Administration)

- **Control:** basal diet without additives
- **YCW:** recommended inclusion rate

❖ Trial Objective

- Assess the effect of **first-feed YCW** on:
 - Early gut microbiota colonisation
 - Immune function across gut segments



Family-level microbial composition across gut segments: early response to YCW

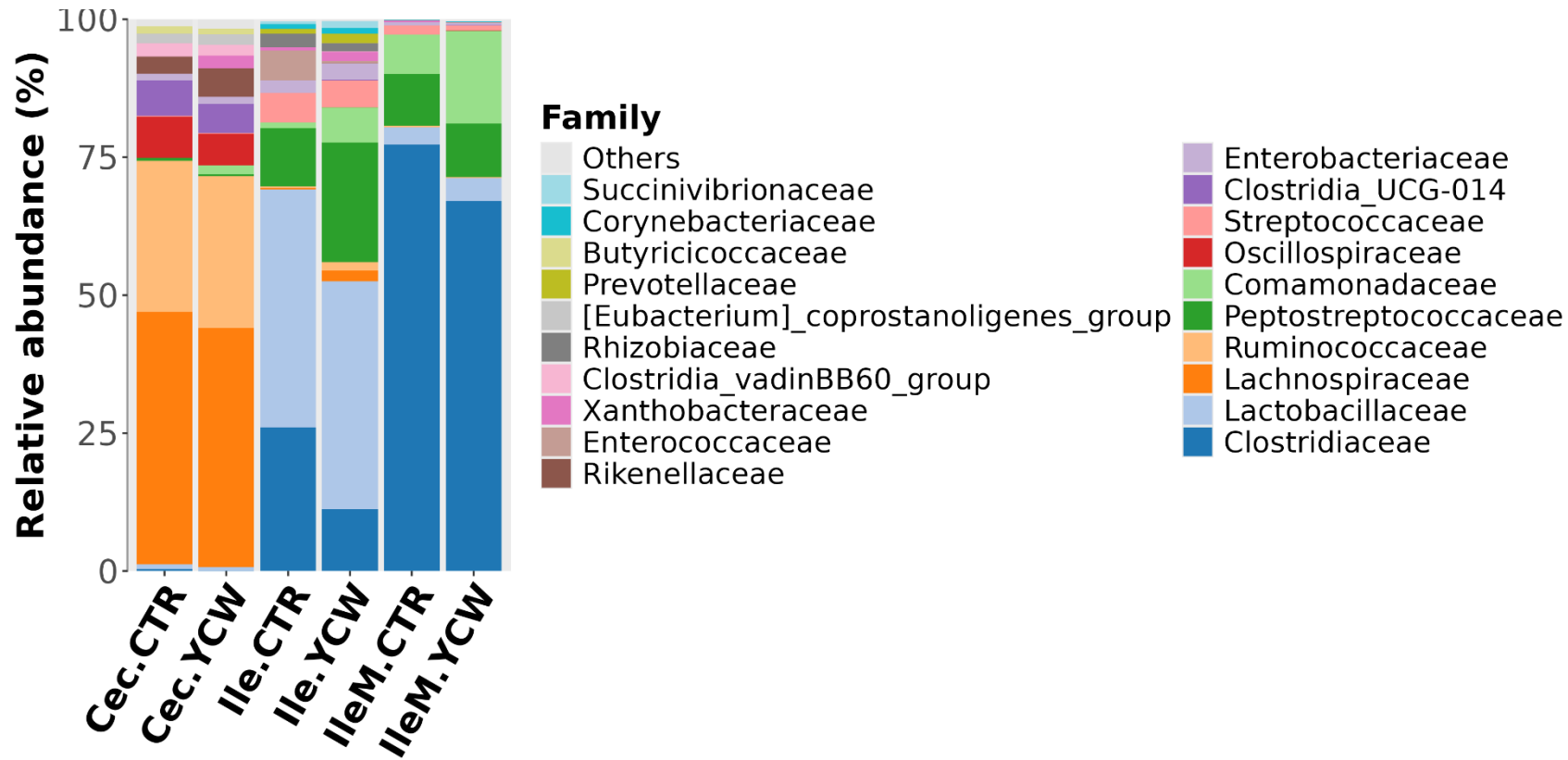


Figure 2. Early YCW supplementation induced significant shifts in key microbial families across gut regions, with increases observed in fermentative and mucosa-associated protective taxa. Differential abundance analyses identified both moderately significant ($P < 0.05$) and highly significant ($P < 0.01$) responses, reflecting early, site-specific modulation of the cecal, ileal, and ileal mucosal microbiota.

Thank you!

