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Optimal gut function in monogastric livestock

# Differences between low and high body weight broilers and strategies for catch up growth of low-performing chickens

**Nadia Everaert**

**Nutrition and Animal Microbiota Ecosystems Lab, KU Leuven – Belgium**

EAAP Annual Meeting, 1/5 September 2024

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

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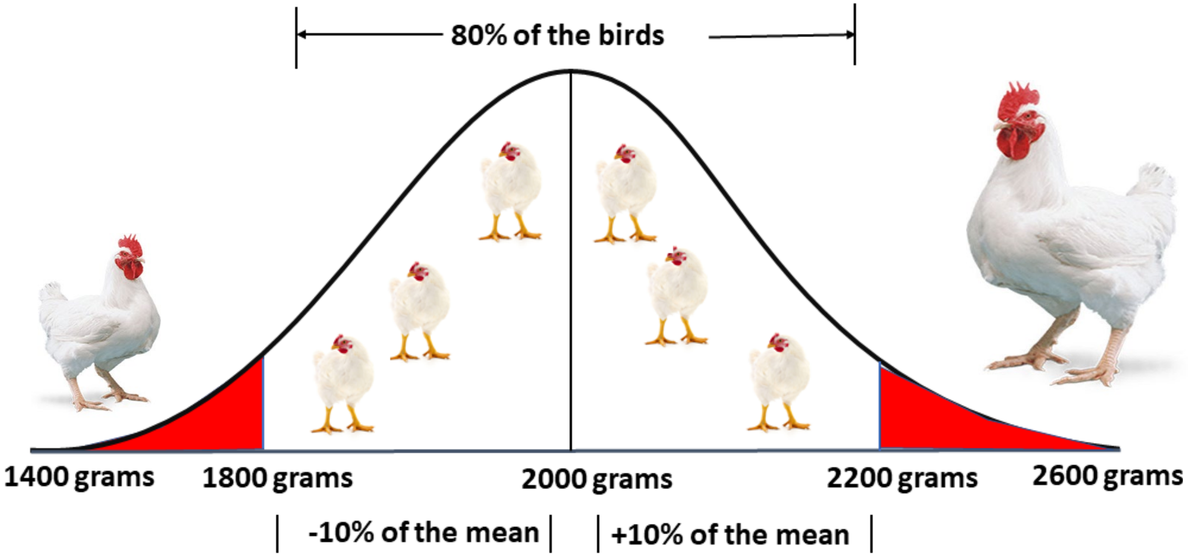
# Introduction

- Intensive selection has reduced the genetic variation in modern broilers and improved their growth performance.
- **Flock weight uniformity:** Percent individuals within a specific range of BW, measures in coefficient of variation (CV). CV is inversely proportional to flock weight uniformity.
- Broiler flocks with **± 8-10% CV** are considered uniform.




Akram et al., 2024; Animal Microbiome



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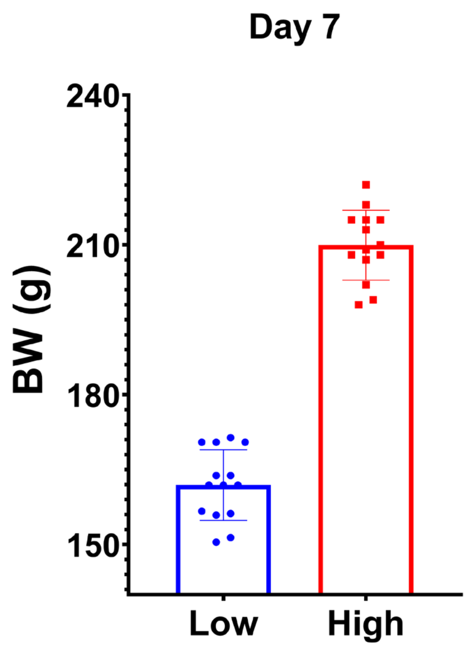
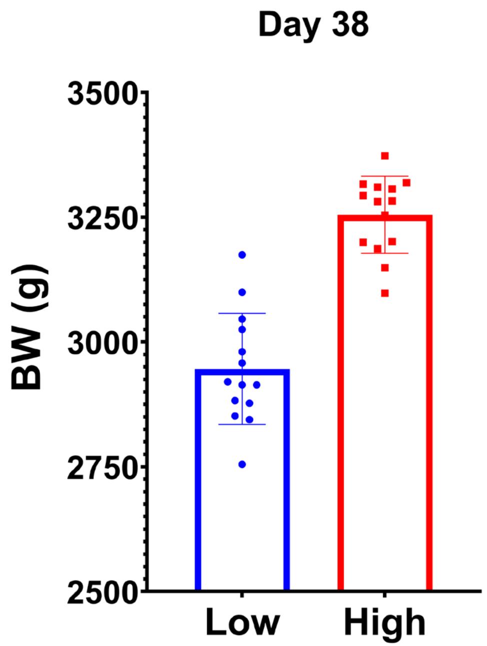
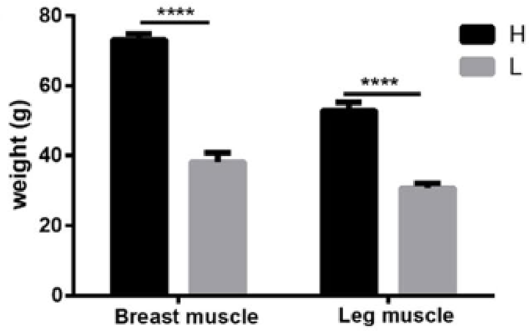
# Introduction

- In commercial settings, broiler chickens have **different growth rates within a flock** under uniform rearing conditions.

- **What are the biological factors causing intra flock body weight (BW) variability?**




Zhang et al. Microbiome (2022)

Akram et al., 2024; Animal Microbiome



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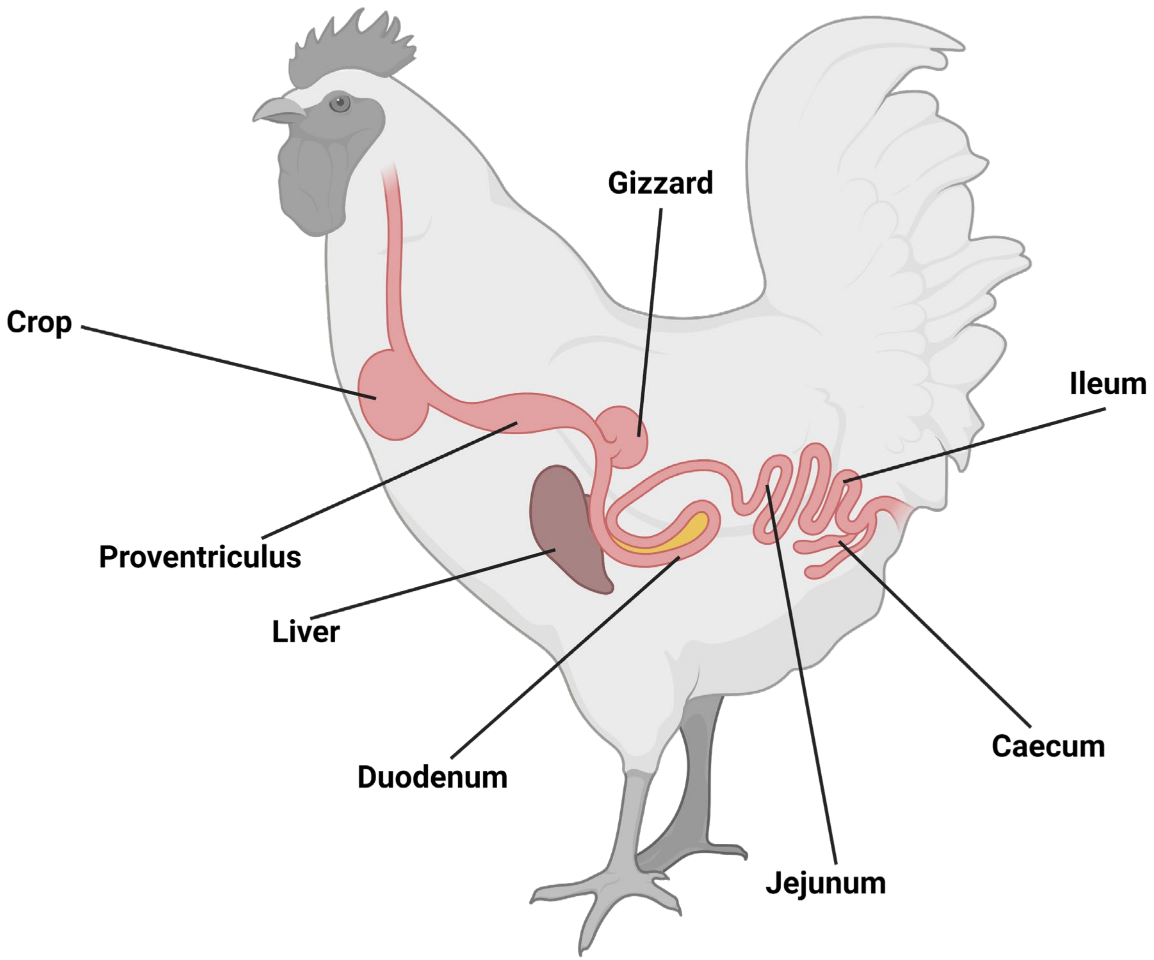
# Introduction

- **Gastrointestinal tract** is more proliferating organ in early life.
- **Early development** of gastrointestinal tract is important for optimal growth and development.
- Helps in meeting **nutritional and immunological demands** in early life.
- Boosts **immune function** and disease resistance.
- Reduces early **mortality and health issues**.

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**Based on** https://doi.org/10.3390/ani11102795

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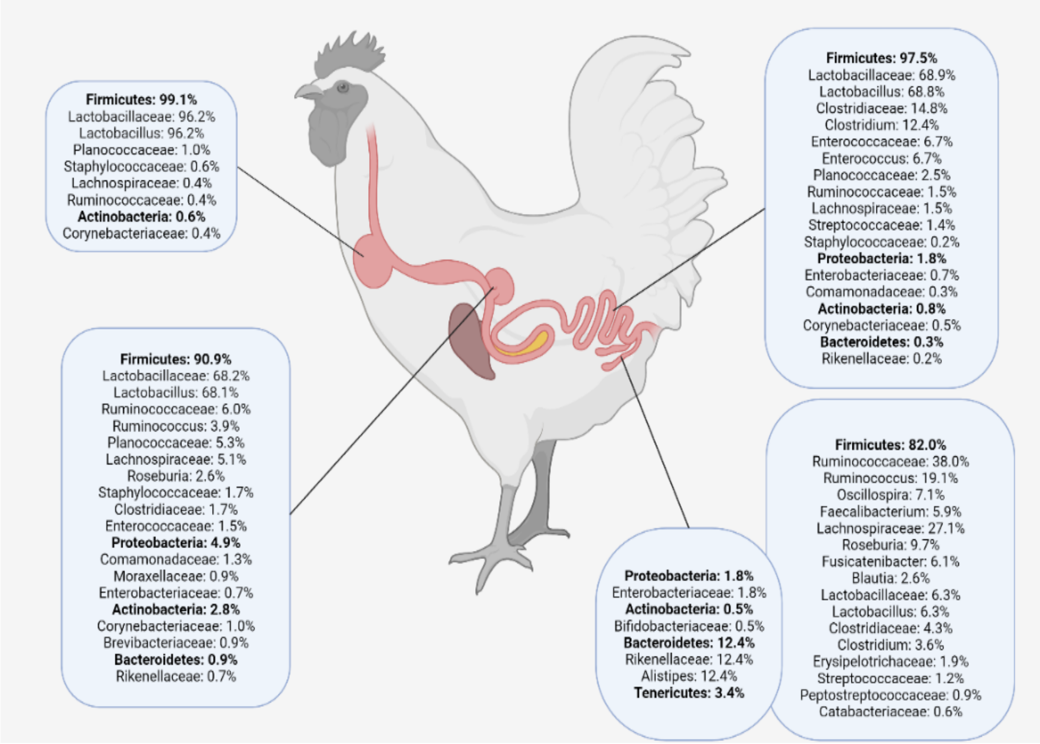
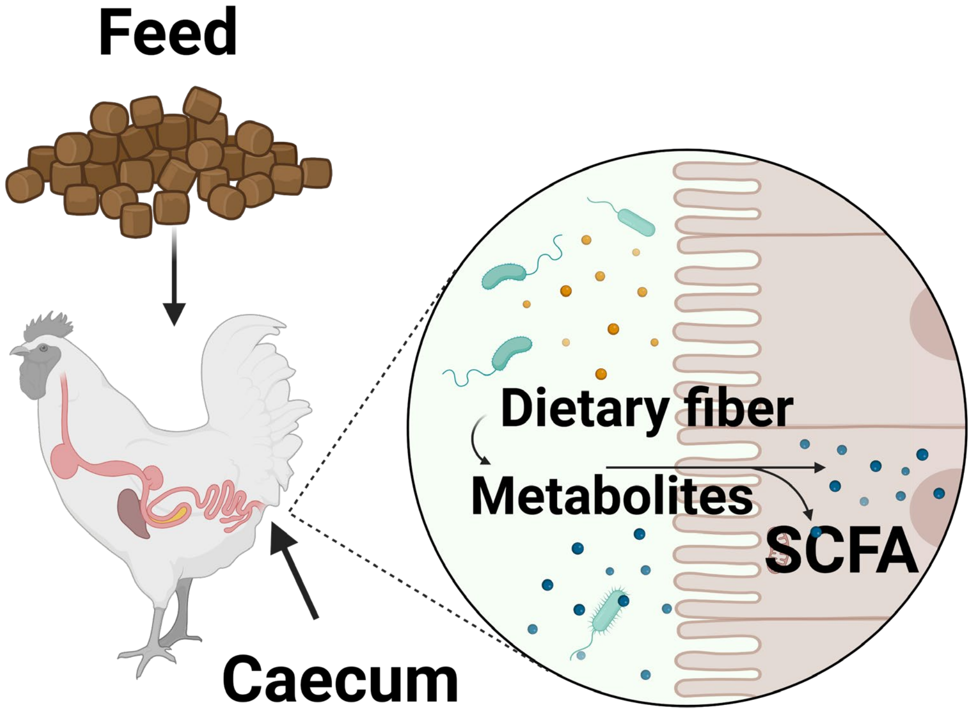
# Introduction

- **Gut microbiota** residing in the digestive tract, contribute to digestion, nutrient absorption, metabolism, and immune regulation

- Intestinal microbiota produces **high-energy metabolites (short-chain fatty acids)** that benefit the host




**Based on** https://doi.org/10.3390/vaccines11030595

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# Introduction

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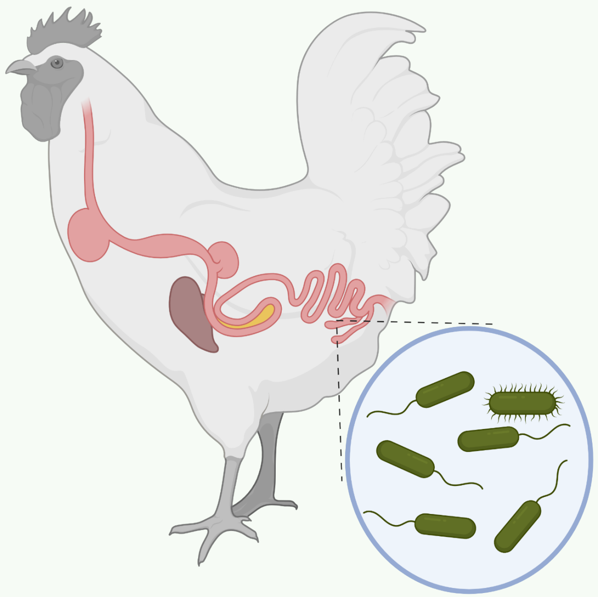
**‘EUBIOSIS’**
Growth and health conferring balanced microbiota community

**‘DYSBIOSIS’**
microbial community involving disbalances or pathologies occur

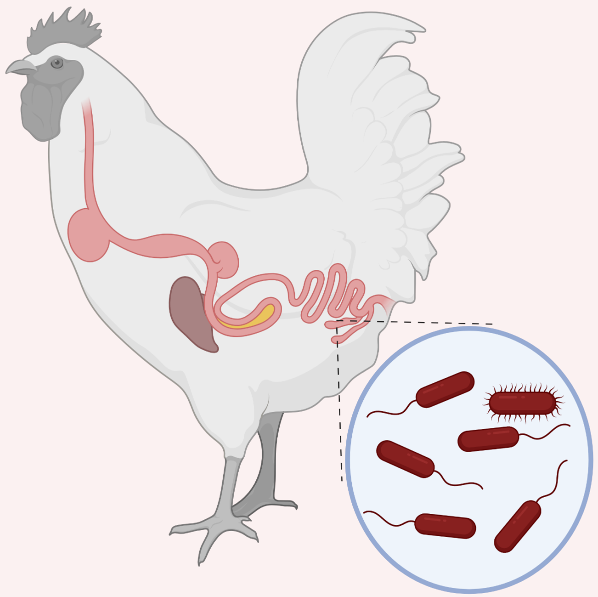
## Eubiosis



↑ Firmicutes to Bacteroidetes ratio
*Lactobacillus*, *Bifidobacterium*, and *Bacteroides*.....

- Reduced Pathogen Load
- Balanced Immune Function

## Dysbiosis



↓ Firmicutes to Bacteroidetes ratio

↑ Campylobacter, Salmonella, and Escherichia......

- Increased Disease Susceptibility
- Retarded growth performance

**Based on** https://doi.org/10.1016/j.aninu.2023.03.005

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# Objectives of the project

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- Characterization of **gastrointestinal related factors** contributing to body weight variation in broiler chicken flocks.
- Development of **supplementary interventions** to improve enteric microbial colonization in lo-performing broiler chicks to improve intestinal health and function.
- Development of **dietary strategies** to improve early life performance and health in low-performing broiler chicks and to reduce flock body weight variations.



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01

# Study 1: Identification of gastrointestinal related factors affecting body weight variability in broiler flocks



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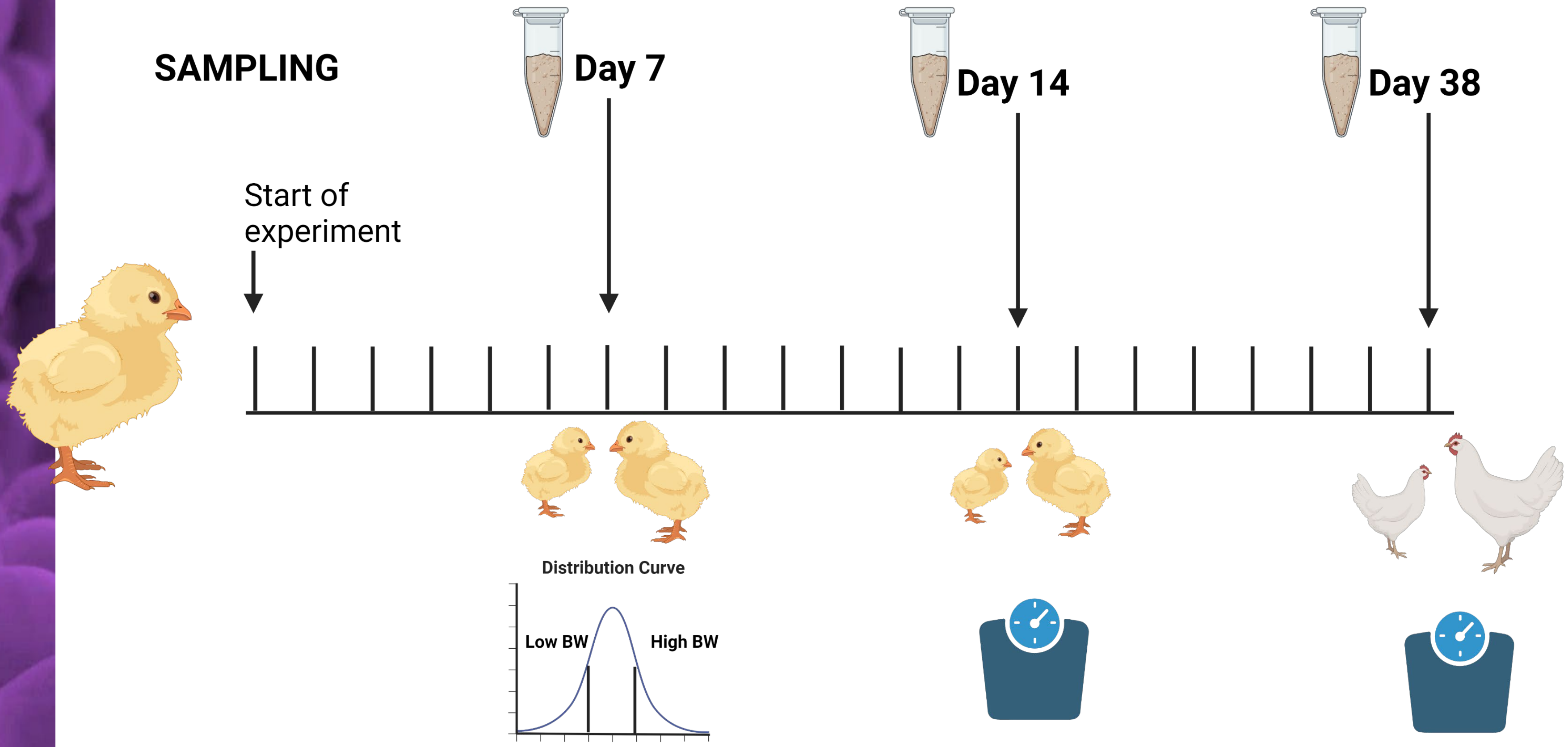
# Trial design




- Ross 308 male broilers
- Floor pens, 21 birds/pen
- 14 replicate pen/BW group
- Bodyweight categorization at day 7

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# Overview of analysis

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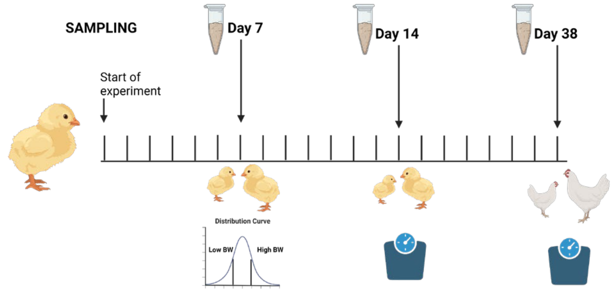
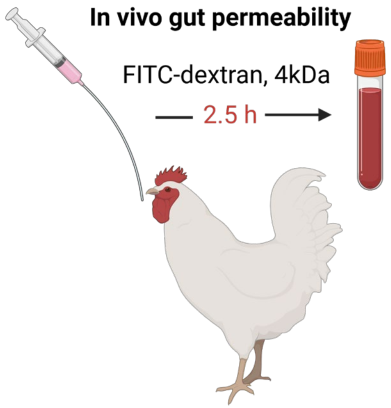
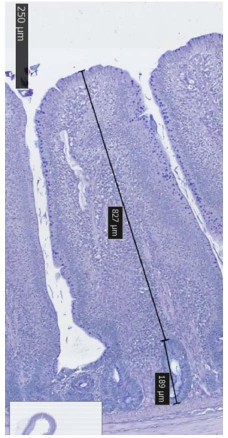
1. Growth performance

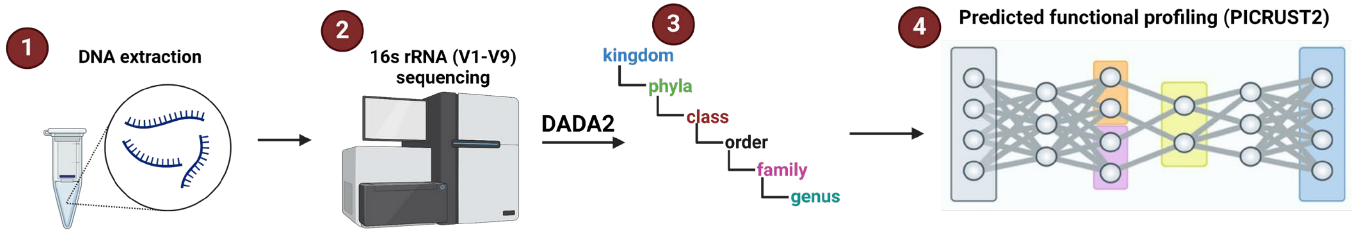



2. *In vivo* permeability, intestinal morphology

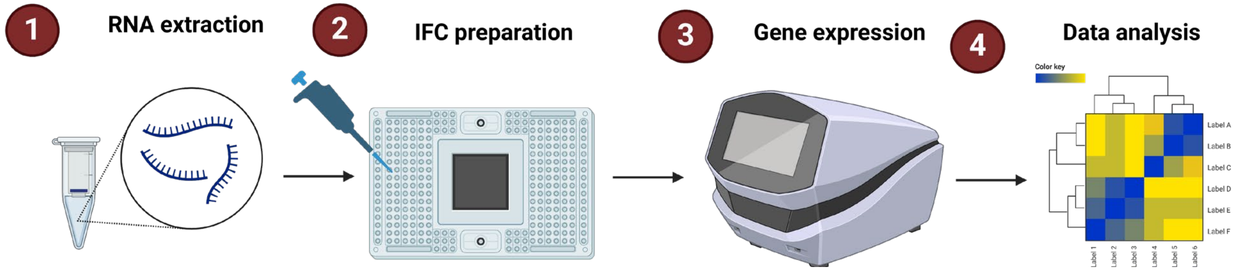


3. Caecal microbiota composition




4. Ileal gene expression

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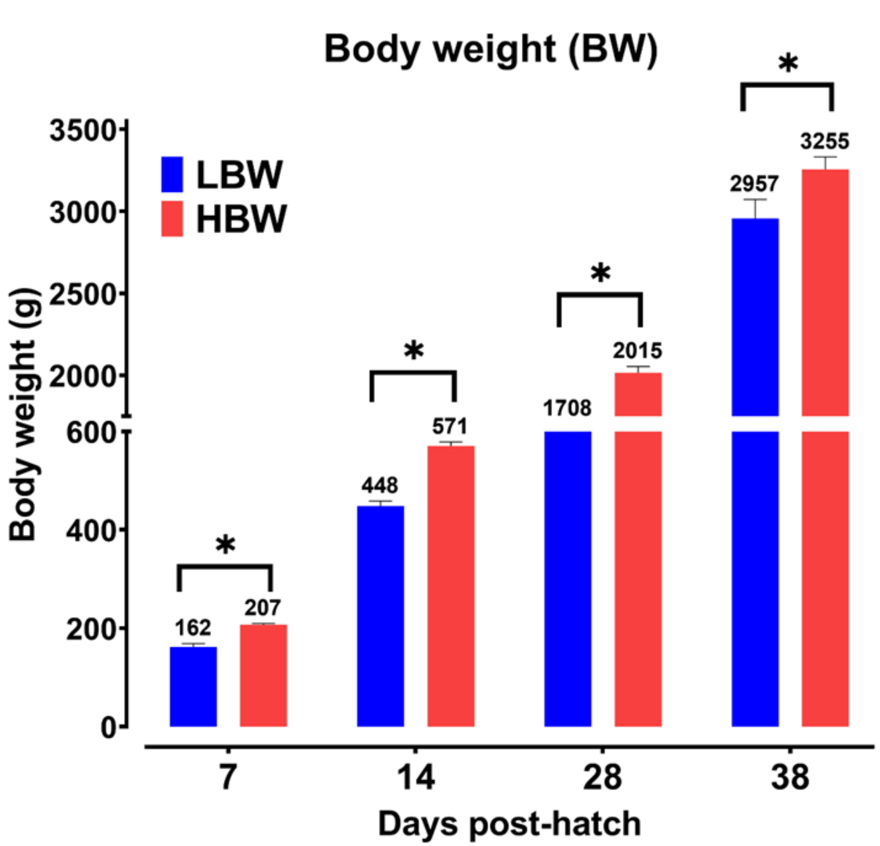
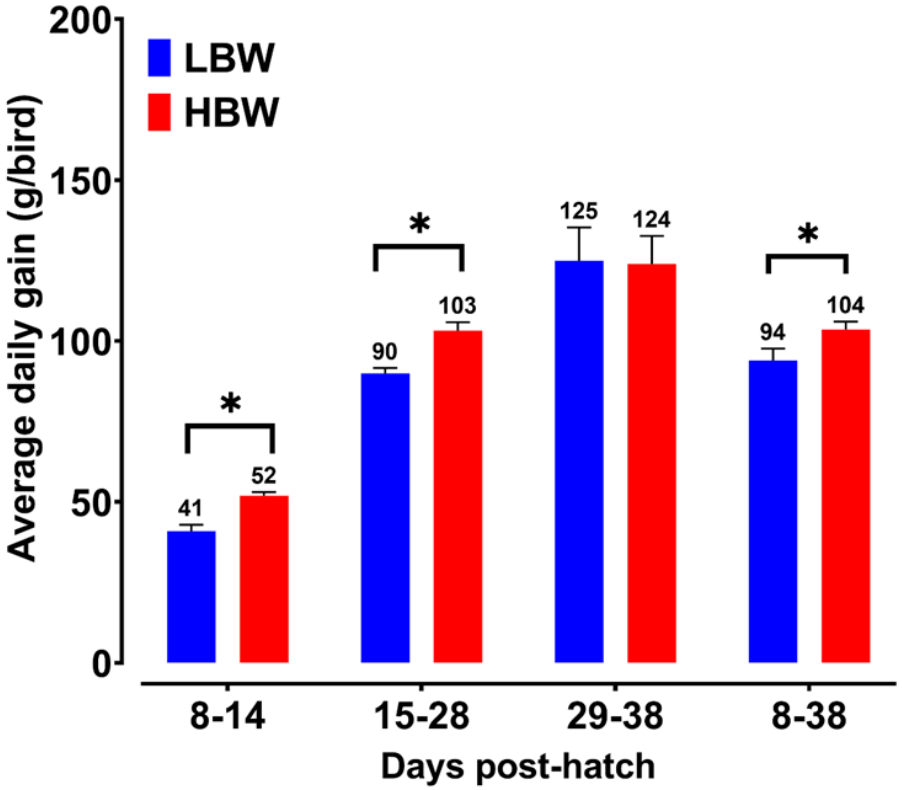
# 1. Growth performance

A




B




Student's t-test, $P < 0.05$



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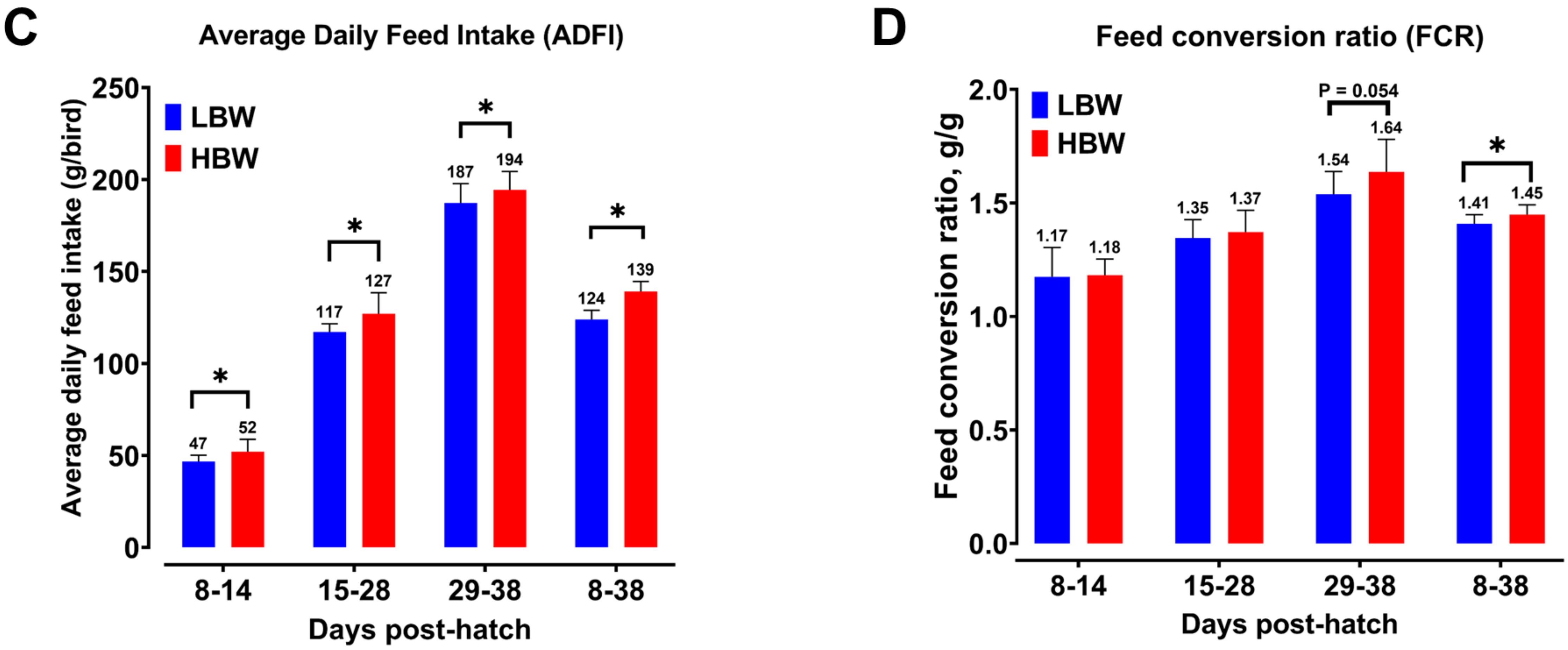
# 1. Growth performance




Student's t-test, P < 0.05



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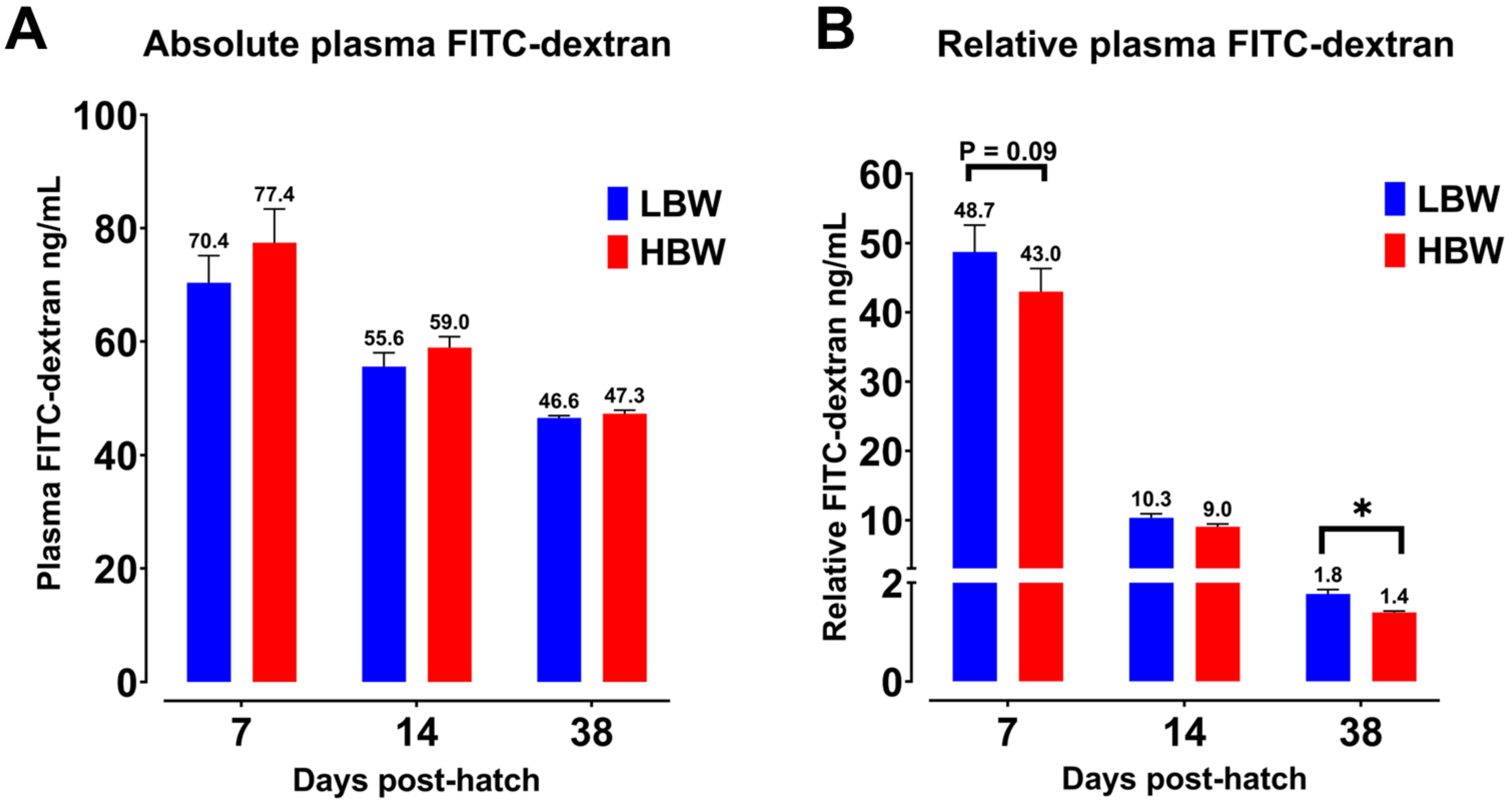
# 2. Intestinal permeability



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# 3. Intestinal histomorphology

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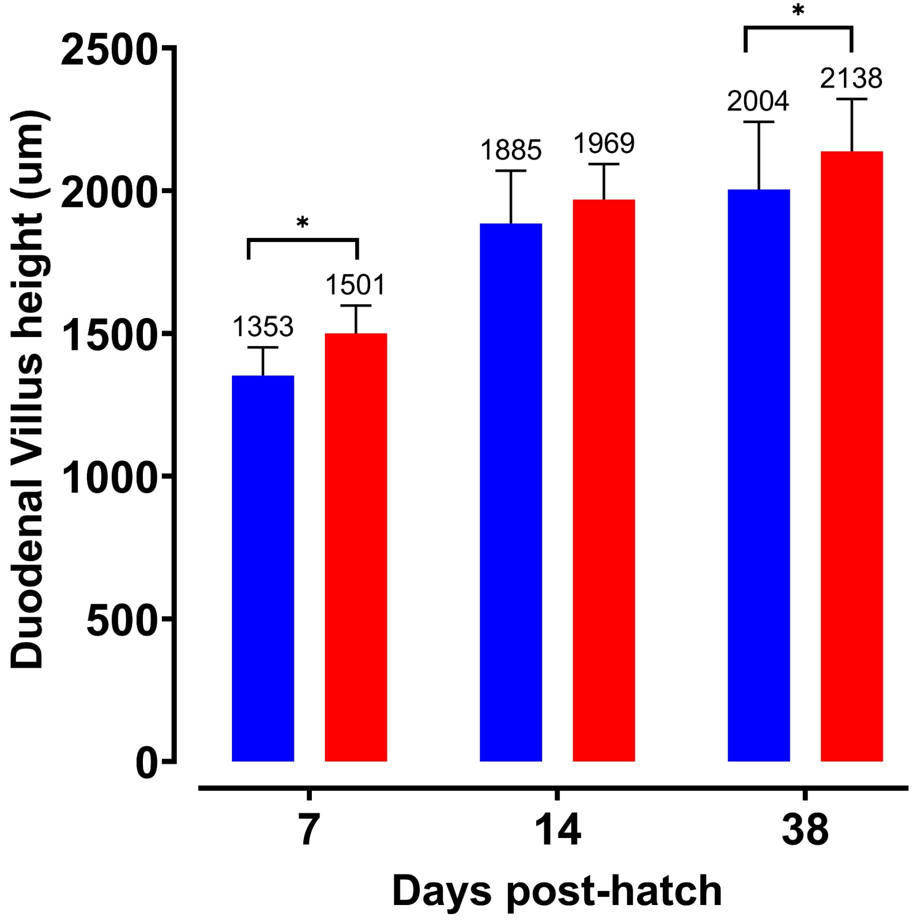
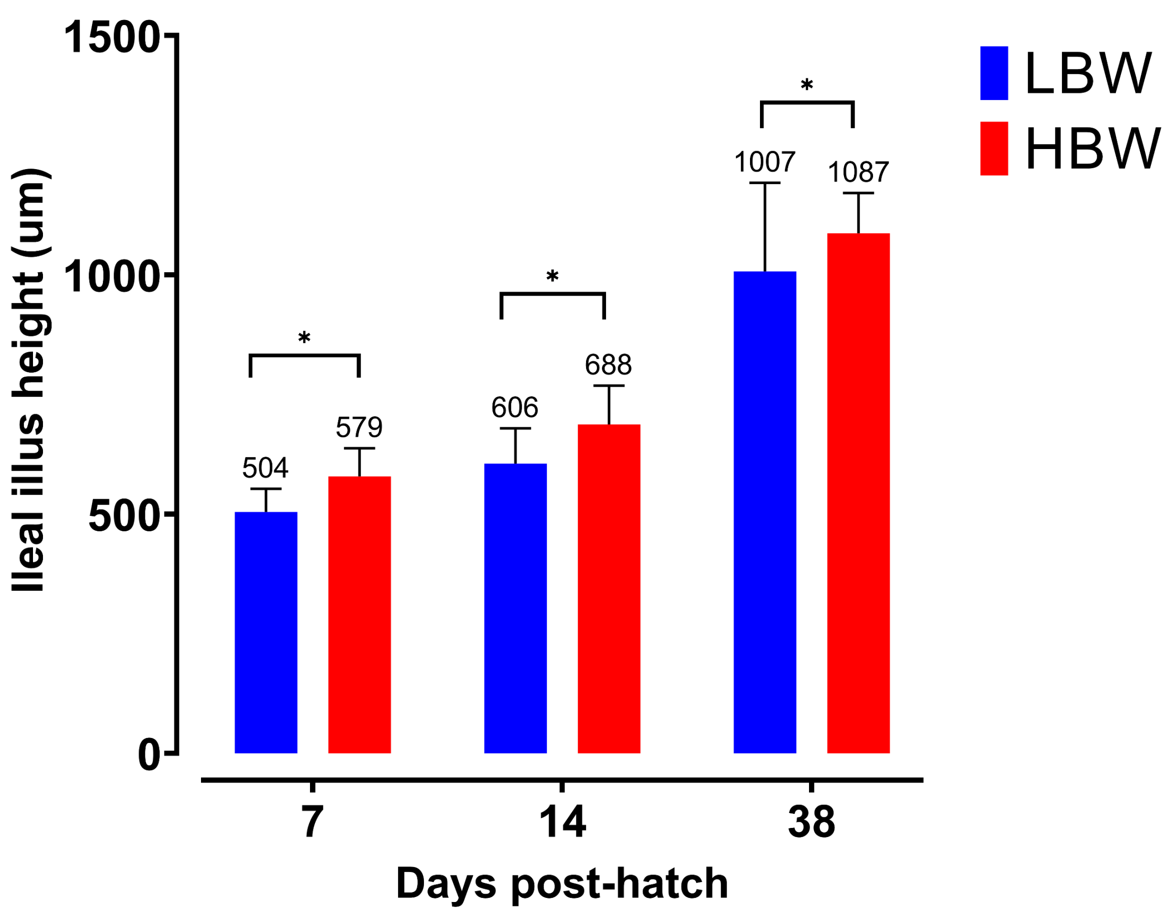
Villus height (Duodenum)




Villus height (Ileum)



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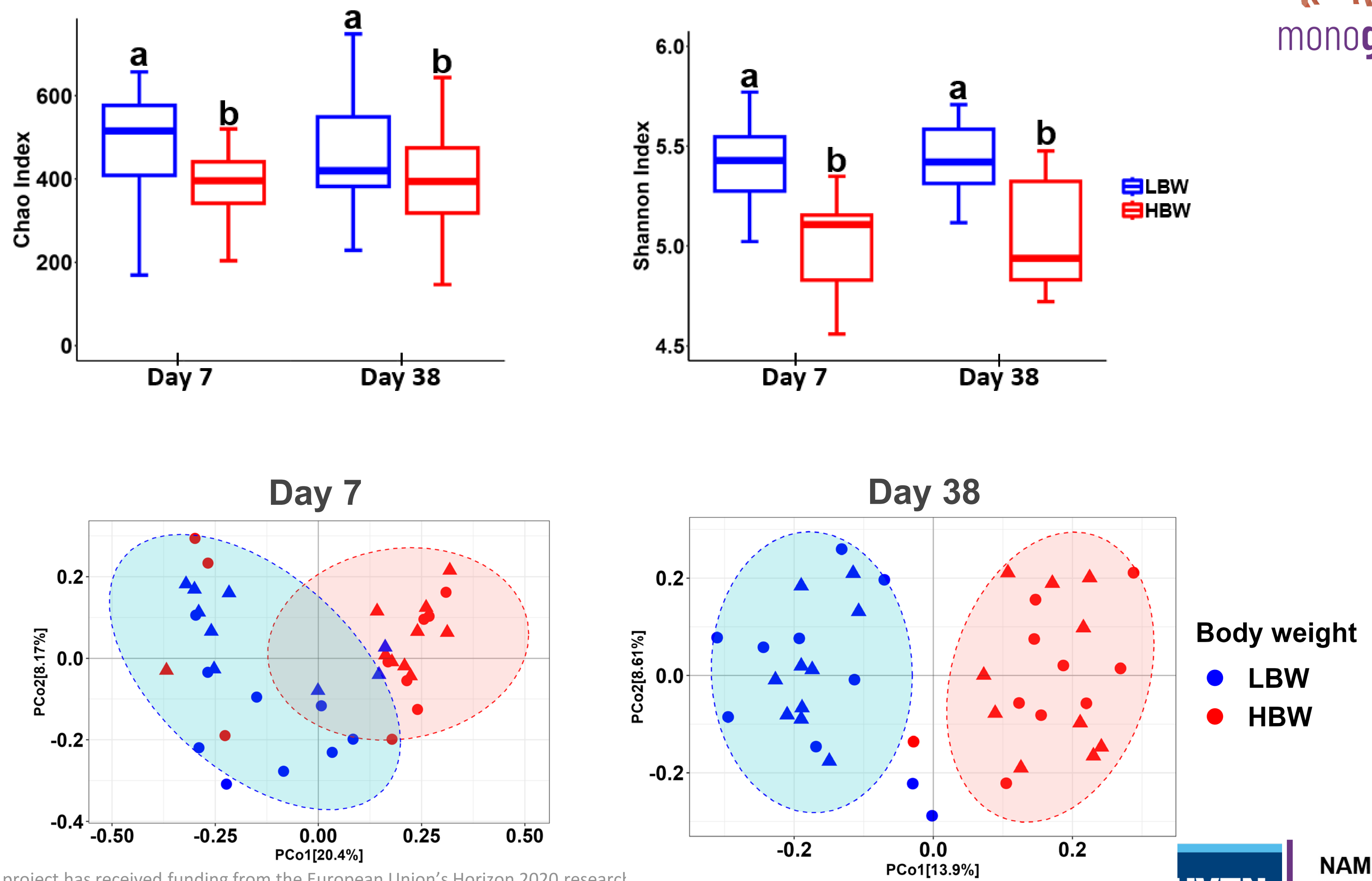
# 3. Caecal Microbiota diversities



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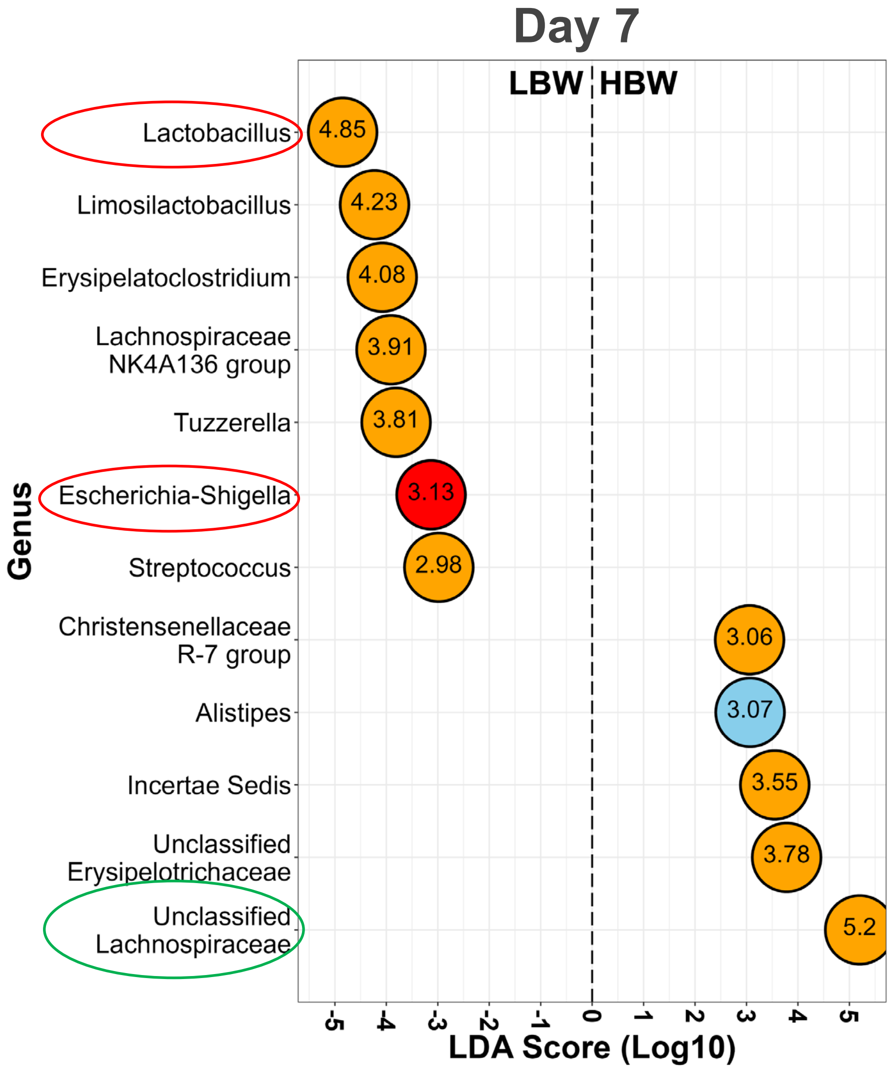
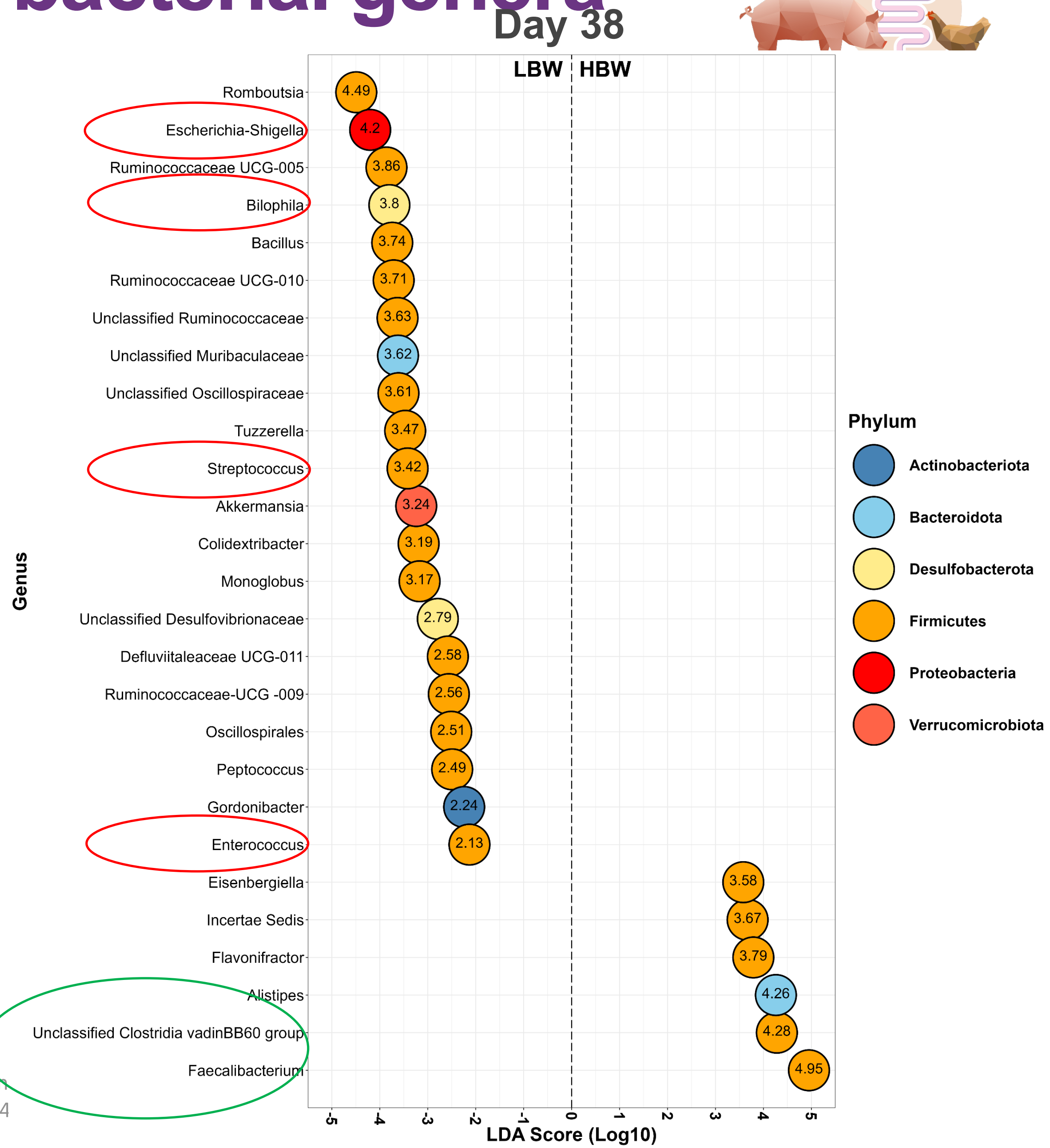
# 3. Differentially abundant bacterial genera




16S rRNA gene sequencing
LEfSe, LDA > 2.0, FDR < 0.05



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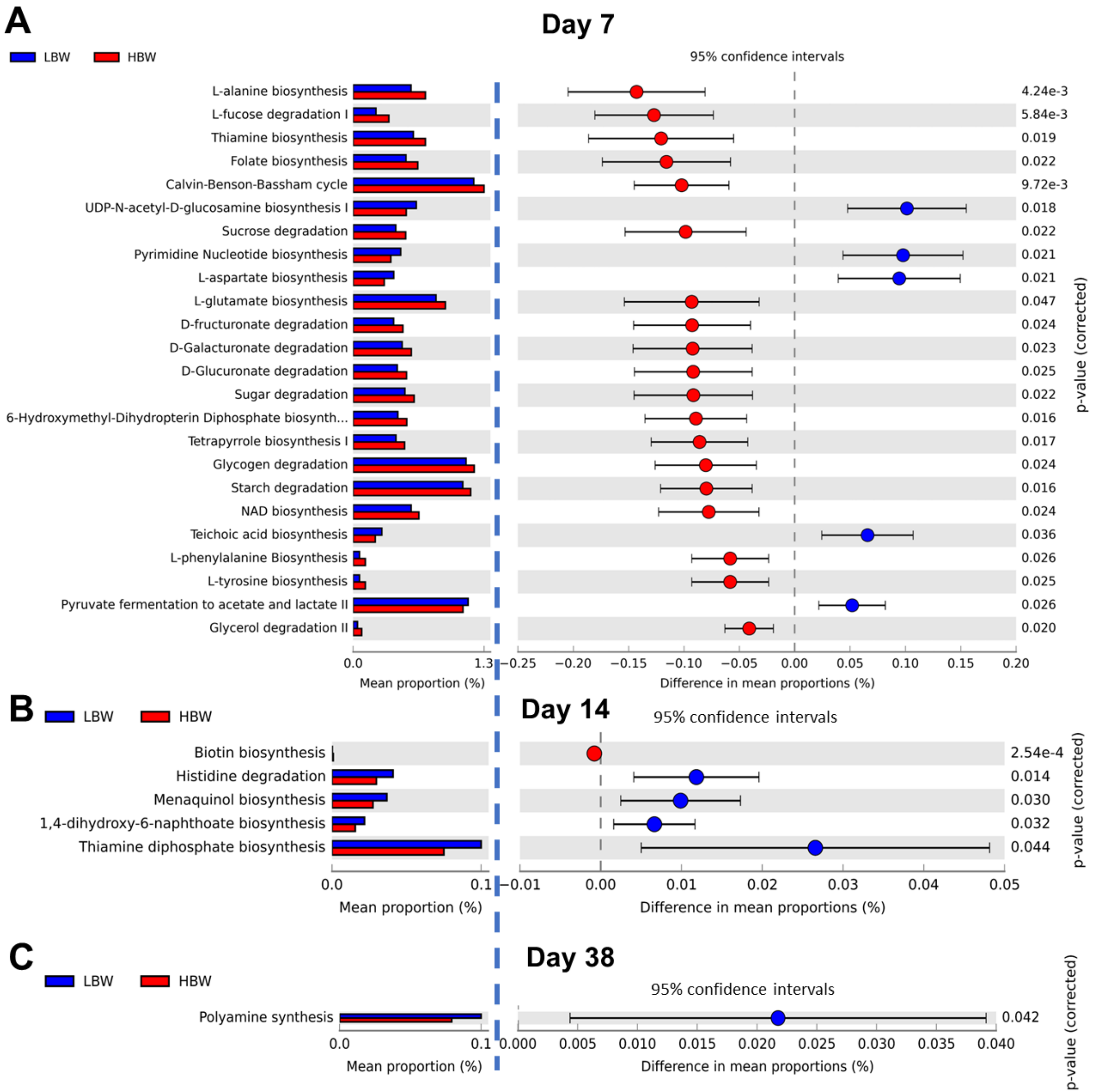
# 3. Predicted functionality – PICRUST2




**HBW:** Microbial pathways enriched in the biosynthesis of amino acids, vitamins and degradation of complex carbohydrates.

**LBW:** Pathways enriched in cell wall antigen (UDP-N-acetyl-D-glucosamine)

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# 4. Ileal gene expression

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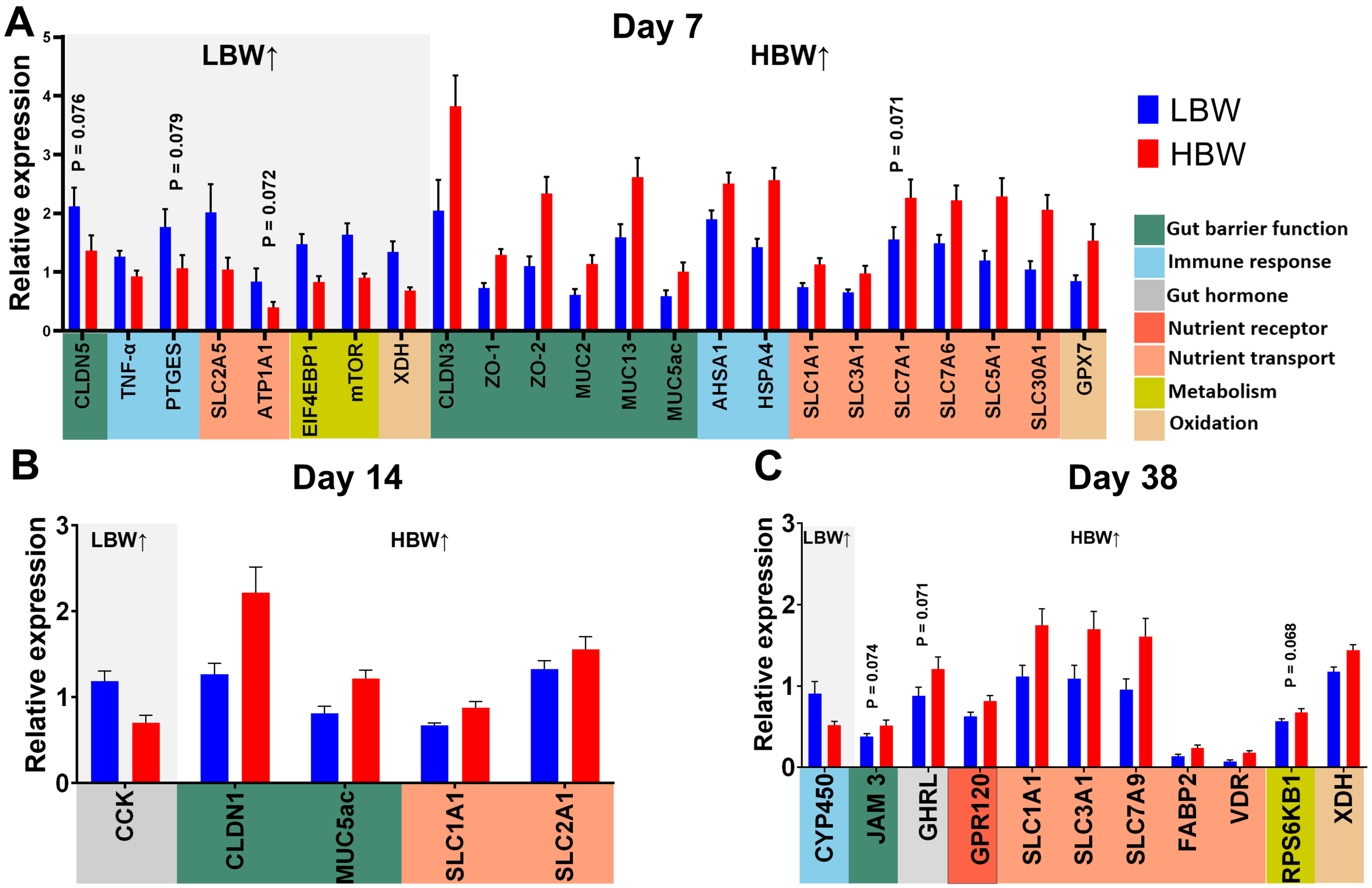


HBW → Barrier function, nutrient transport and antioxidant genes ↑

LBW → Immune response and neuropeptide hormone CCK ↑

Student's t-test, P < 0.05



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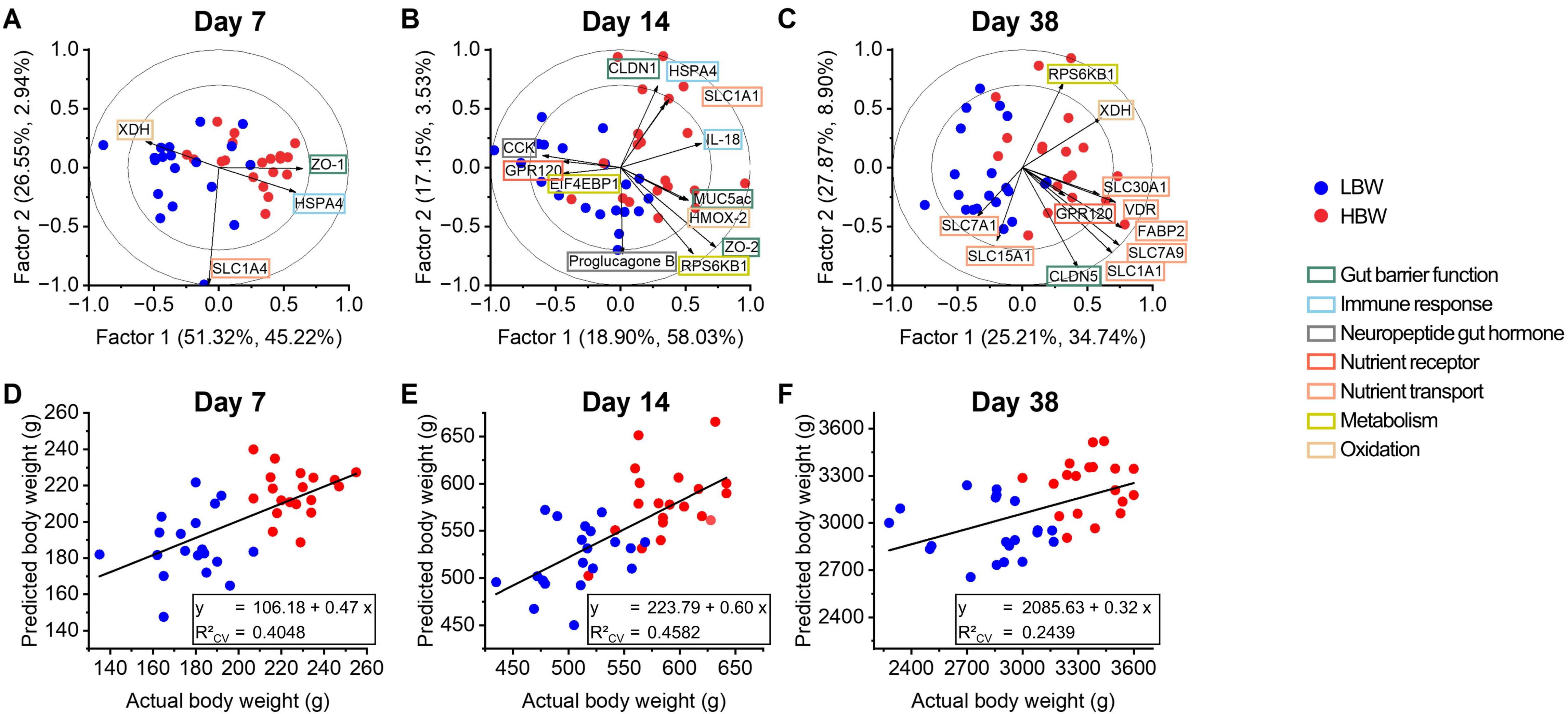
# 4. Ileal gene expression – Partial least square regression

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# Conclusion

- Broiler chickens within a flock shows **divergence growth rates** despite uniform rearing conditions.
- **Differential gene expression** was observed, mainly related to nutrient transport, absorption and oxidation, and intestinal barrier function.
- **Feed intake** seemed to be controlled by **gut hormones**, which led to differences in weight gain.
- Variations in the **caecal microbiota composition and functionality** were observed starting from 7 days, coinciding with the divergence of performance.



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# For further reading

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## Animal Microbiome

Research | Open access | Published: 24 July 2024

### Assessing the impact of hatching system and body weight on the growth performance, caecal short-chain fatty acids, and microbiota composition and functionality in broilers

Muhammad Zeeshan Akram, Ester Arévalo Sureda, Luke Comer, Matthias Corion & Nadia Everaert

*Animal Microbiome* **6**, Article number: 41 (2024) | Cite this article

**546** Accesses | **1** Citations | Metrics

ELSEVIER

## Poultry Science

Available online 2 August 2024, 104158

In Press, Journal Pre-proof | What's this?

### Linking gastrointestinal tract structure, function, and gene expression signatures to growth variability in broilers: a novel interpretation for flock uniformity

Muhammad Zeeshan Akram * †, Ester Arévalo Sureda *, Matthias Corion *, Luke Comer *, Nadia Everaert *

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https://doi.org/10.1016/j.psj.2024.104158

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02

# Study 2: Effects of in ovo sodium butyrate (SB) injection on growth performance, intestinal health and gut microbiota of chickens with varying hatch weight (HW)



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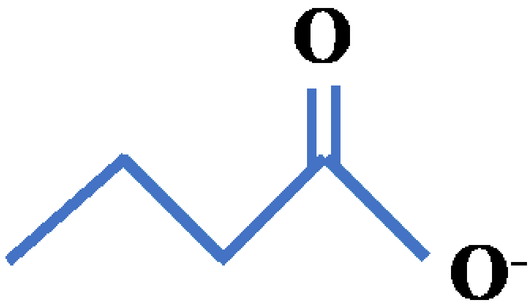
# Rationale for choosing sodium butyrate (SB)?

- Butyrate, a **major SCFA** produced by bacterial fermentation of undigested carbohydrate in the gut.
- It acts as energy source for enterocytes, improve **gut barrier function.**
- It enhance **mucin production**, which might modulate gut microbiota.
- It has been shown to increase **digestive and absorptive functions** of the intestine.
- It modulates immune function, improves intestinal health by reducing the intestinal **inflammatory response**.




Butyrate

**Based on** https://doi.org/10.1016/j.psj.2024.104108



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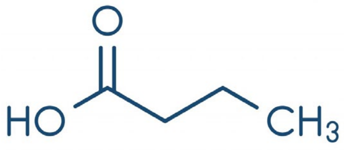
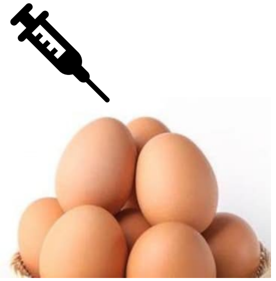
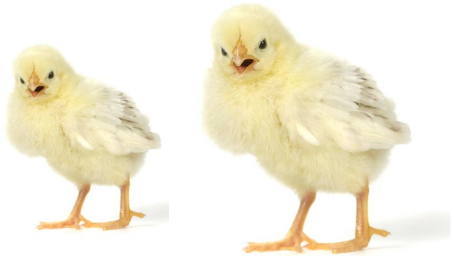
# Objectives and Aim




butyric acid





**Phenotype of chickens**

To examine the effect of in-ovo SB injection on growth performance, intestinal development and gut microbiota of chickens with different hatch weight (HW)



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# Trial design

## 4 x 2 factorial design

- Control: normal saline (n=300)
  - LHW-control
  - HHW-control
- SB1: 0.1% sodium butyrate (n=300)
  - LHW-SB1
  - HHW-SB1
- SB3: 0.3% sodium butyrate (n=300)
  - LHW-SB3
  - HHW-SB3
- SB5: 0.5% sodium butyrate (n=300)
  - LHW-SB5
  - HHW-Sb5l

## Analyses (n = 6 birds/group)

- Relative intestinal weight and length
- Expression of gene related to gut barrier function and immune response (qPCR)
- Caecal microbiota feature including predicted metabolic pathways (16S sequencing)

**Day 0**
Start incubation

**Day 12**
In ovo injection in air sac (0.2 mL)

**Day 1**
Hatch day LHW and HHW categories

**Day 14**
First sampling

**Day 35**
Body weight and feed intake

**Day 42**
Second sampling

Pre-hatch period

Post-hatch period

thealth

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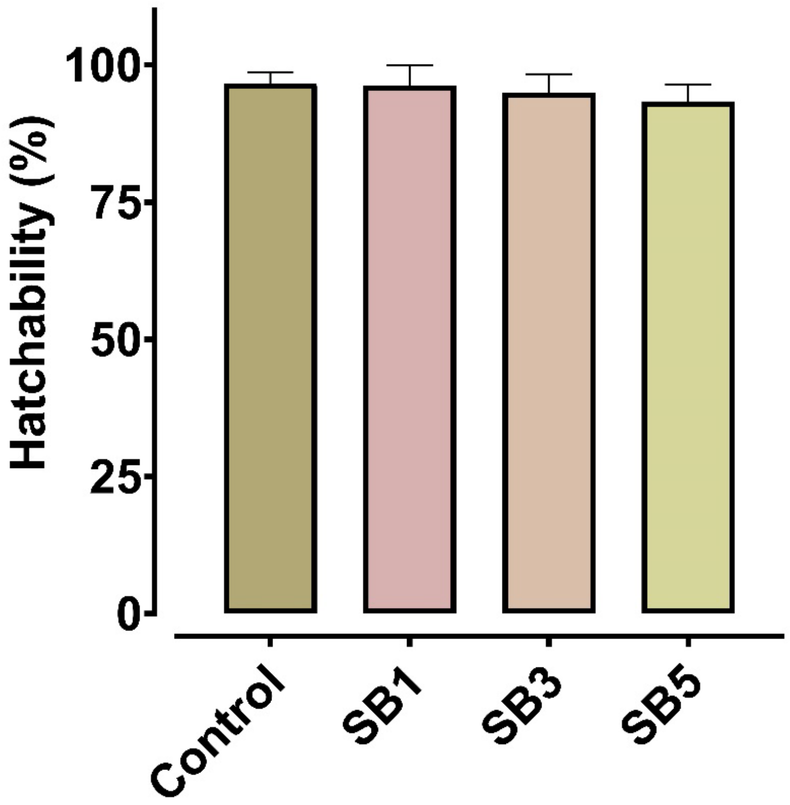
# SB effects on embryonic development




- No negative effects on **embryonic development**
- No effect on **hatchability**

**Control:** 0% SB**, SB1:** 0.1% SB**, SB3:** 0.3% SB**, SB5:** 0.5% SB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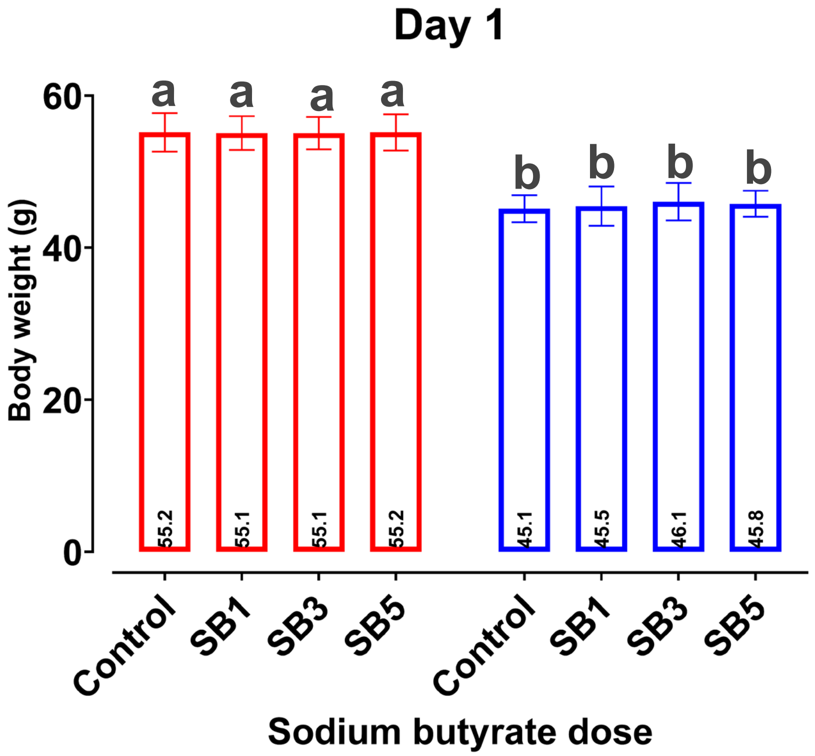
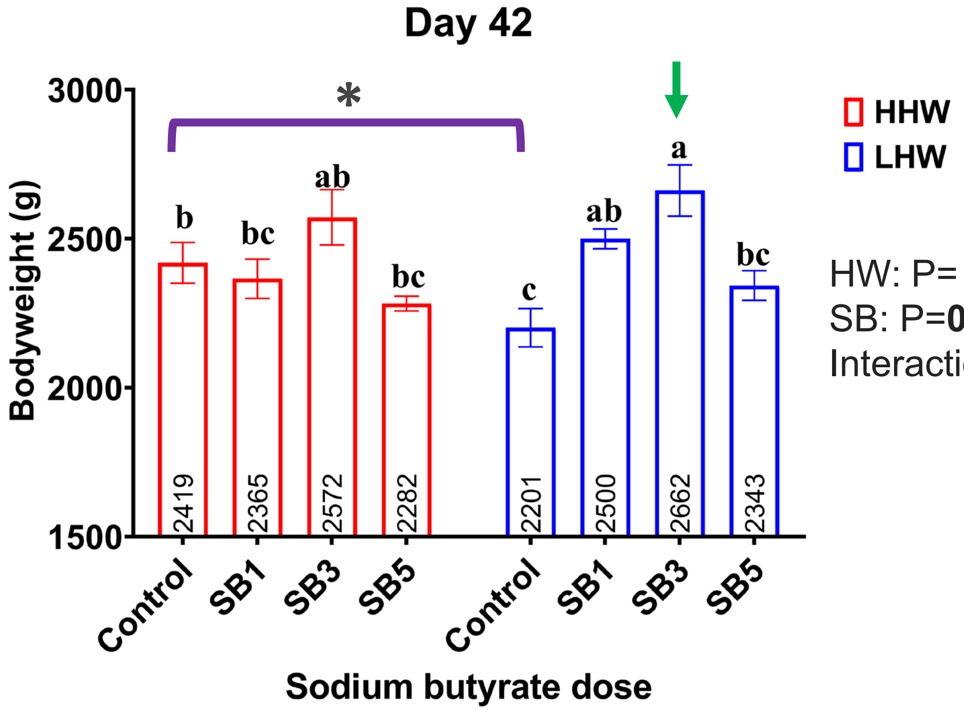
# 2. Body weight




HW: **P<0.001**
SB: P=NS
Interaction: P= NS




HW: P= NS
SB: P=**0.009**
Interaction: P= **0.045**

abc: TukeyHSD, P<0.05
**Control:** 0% SB**, SB1:** 0.1% SB**, SB3:** 0.3% SB**, SB5:** 0.5% SB



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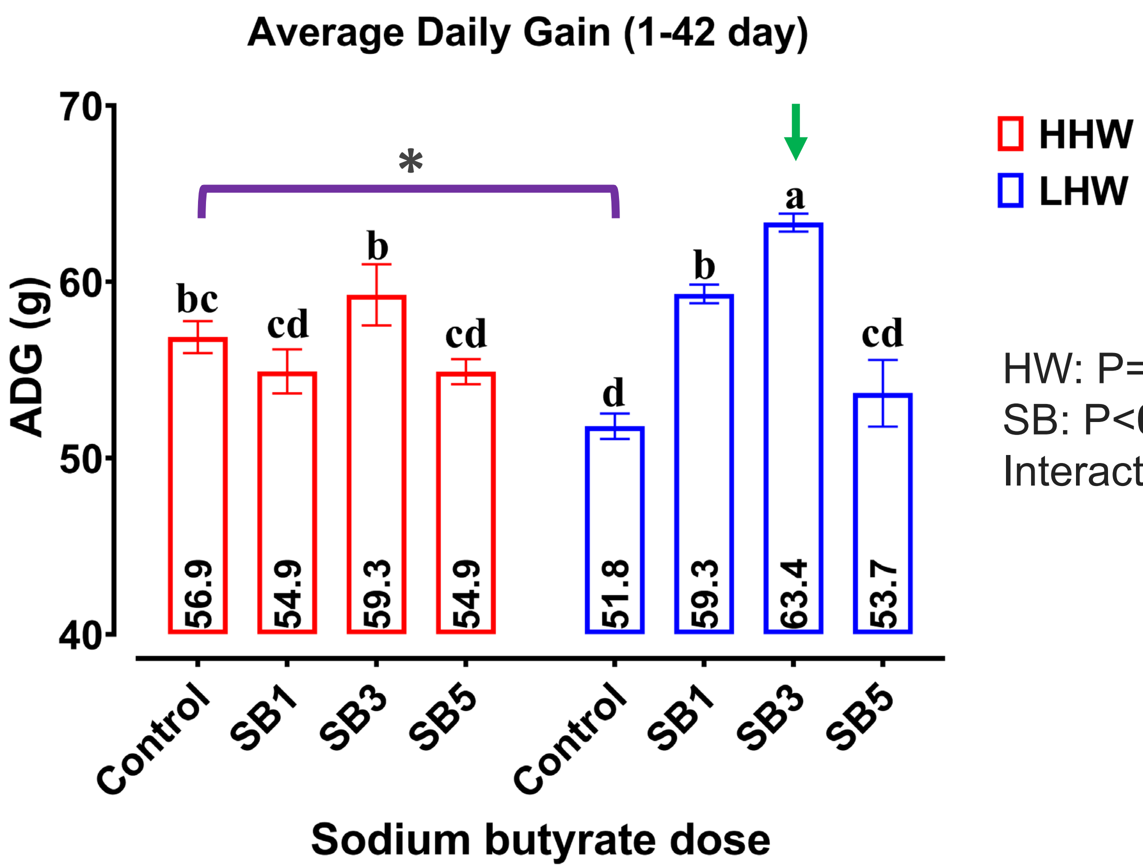
# 2. Average daily gain




HW: P= NS
SB: P<**0.001**
Interaction: P< **0.001**

abc: TukeyHSD, P<0.05

**Control:** 0% SB**, SB1:** 0.1% SB**, SB3:** 0.3% SB**, SB5:** 0.5% SB



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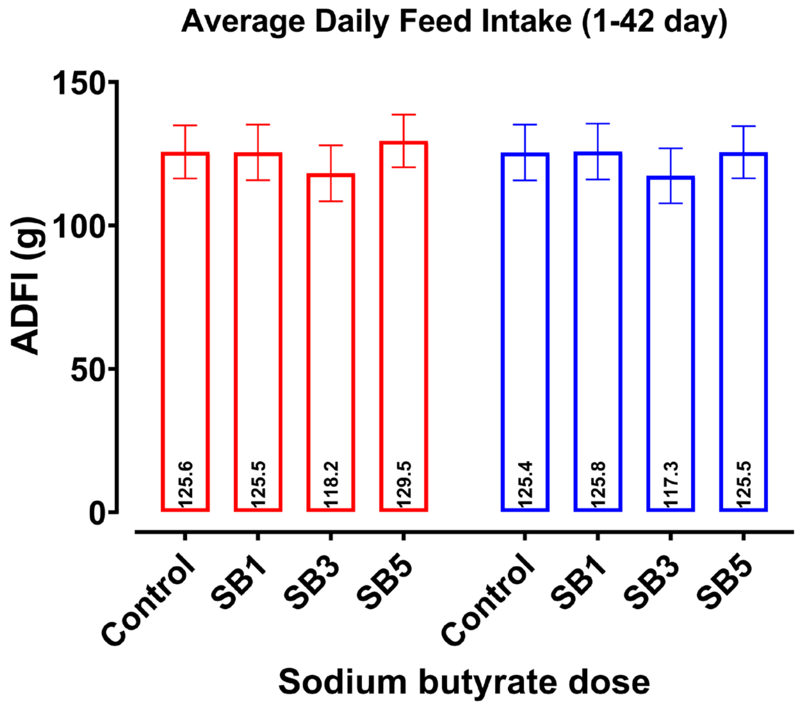
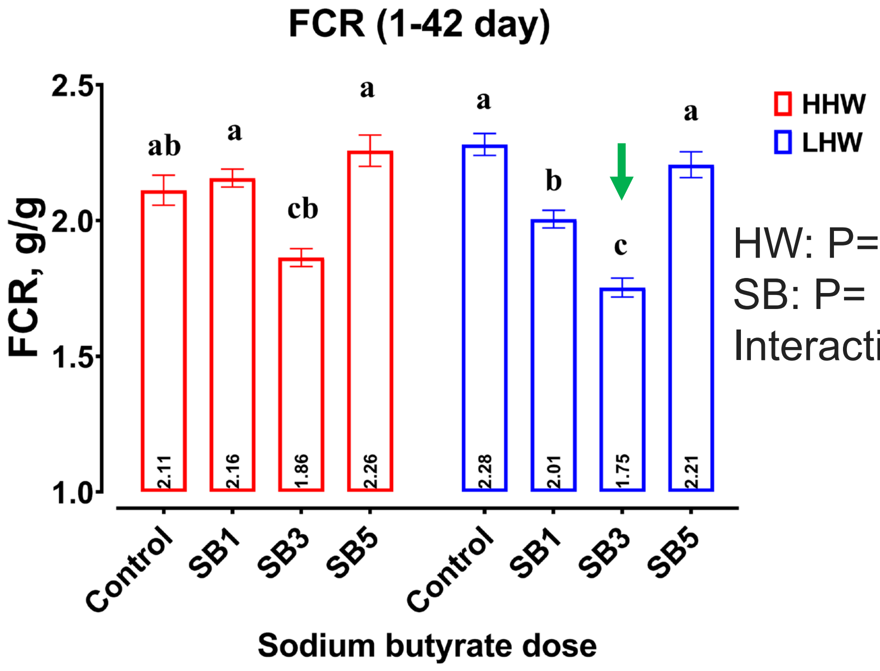
# 2. Feed intake and Feed conversion ratio




HW: P= NS
SB: P= 0.031
Interaction: P= NS




HW: P= NS
SB: P= 0.004
Interaction: P= **0.034**

abc: TukeyHSD, P<0.05
**Control:** 0% SB**, SB1:** 0.1% SB**, SB3:** 0.3% SB**, SB5:** 0.5% SB



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# 3. Ileal gene expression on day 14

| Genes | HHW | | | | LHW | | | | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Control | SB1 | SB3 | SB5 | Control | SB1 | SB3 | SB5 | HW x SB |
| CLDN1 | 1.00[c] | 2.40[ab] | 2.56[ab] | 2.61[ab] | 0.69[d] | 2.49[ab] | $\boxed{3.31^{a}}$ | 1.24[c] | **0.016** |
| TJP1 | 1.00[b] | 2.06[ab] | 1.69[ab] | 2.03[ab] | 1.90[ab] | 2.03[ab] | $\boxed{2.56^{a}}$ | 1.56[ab] | **0.028** |
| MUC6 | 1.00 | 3.05 | 3.09 | 3.61 | 2.15 | 3.29 | 3.58 | 4.76 | 0.633 |
| IL-10 | 1.00[c] | 2.38[ab] | 2.44[ab] | 2.52[ab] | 2.51[ab] | 2.53[ab] | $\boxed{3.01^{a}}$ | 2.10[b] | **0.048** |
| IL-1B | 1.00 | 1.55 | 1.95 | 0.99 | 1.71 | 2.07 | 1.78 | 1.19 | 0.658 |
| IL-12P40 | 1.00 | 1.72 | 1.56 | 1.51 | 2.06 | 1.91 | 1.46 | 1.30 | 0.205 |

abcd: TukeyHSD, P<0.05

**CLDN1**: claudin1, **TJP1**, tight junction protein1, **MUC6**: mucin 6
**IL-10**: interleukin 10, **IL1B**: interleukin 1-beta, **IL-12P40**: interleukin 12 subunit 40



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

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# 3. Ileal gene expression on day 42

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| Genes | HHW | | | | LHW | | | | P-value |
|---|---|---|---|---|---|---|---|---|---|
| | Control | SB1 | SB3 | SB5 | Control | SB1 | SB3 | SB5 | HW x SB |
| CLDN1 | 1.00[ab] | 0.60[bc] | 0.30[c] | 0.52[bc] | 1.01[a] | $\boxed{1.27^{a}}$ | 0.52[bc] | 0.23[c] | **0.002** |
| TJP1 | 1.00 | 0.77 | 0.64 | 0.46 | 1.60 | 1.12 | 0.79 | 0.32 | 0.180 |
| MUC6 | 1.00[ab] | 0.41[b] | 0.40[b] | 0.36[b] | 1.03[ab] | 0.51[b] | $\boxed{1.42^{a}}$ | 0.14[c] | **0.024** |
| IL-10 | 1.00 | 0.46 | 1.18 | 0.53 | 0.86 | 0.93 | 0.41 | 0.58 | 0.207 |
| IL-1B | 1.00 | 1.03 | 0.57 | 0.93 | 0.70 | 1.0 | 0.58 | 0.28 | 0.227 |
| IL-12P40 | 1.00 | 0.55 | 0.96 | 1.01 | 0.92 | 1.27 | 0.80 | 0.48 | 0.109 |

abc: TukeyHSD, P<0.05

**CLDN1**: claudin1, **TJP1**, tight junction protein1, **MUC6**: mucin 6
**IL-10**: interleukin 10, **IL1B**: interleukin 1-beta, **IL-12P40**: interleukin 12 subunit 40



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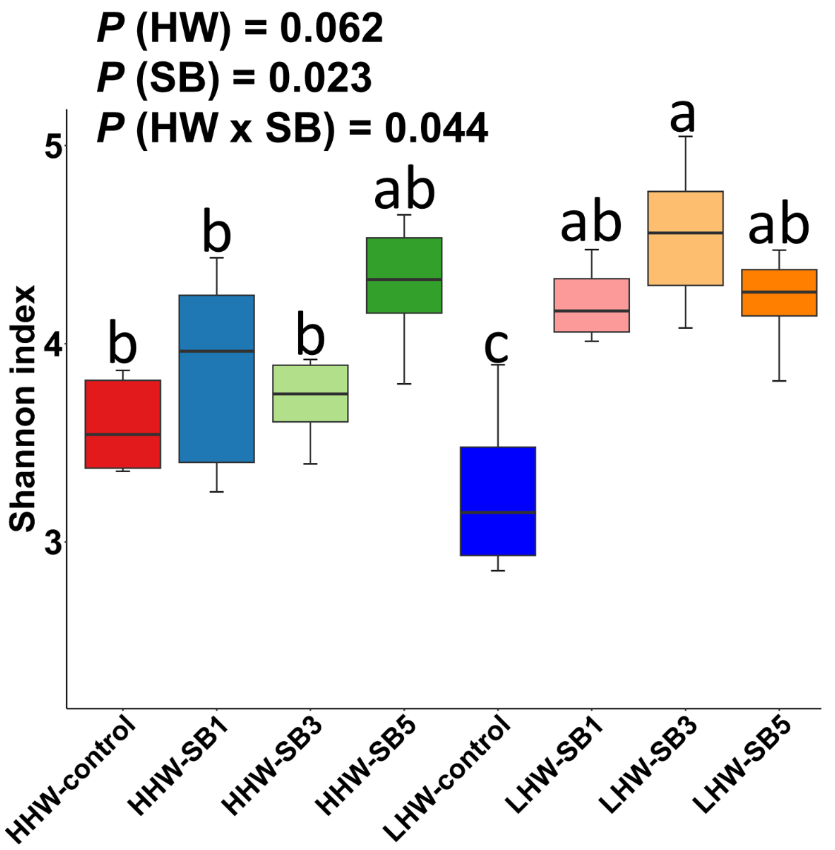
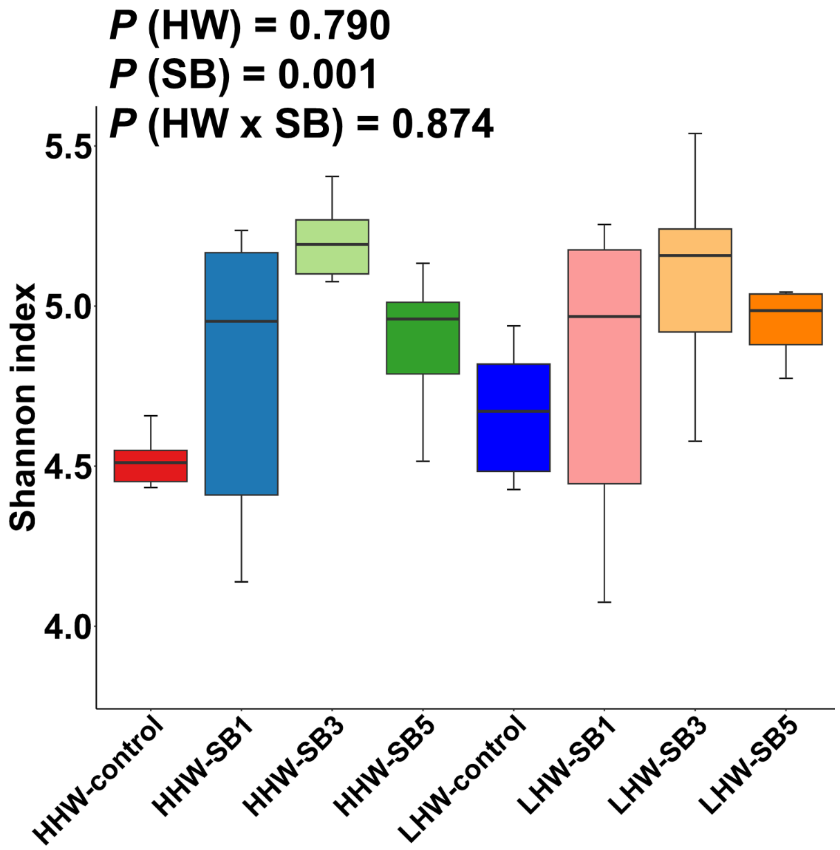
# 4. Caecal microbiota alpha diversity

Day 14




Day 42

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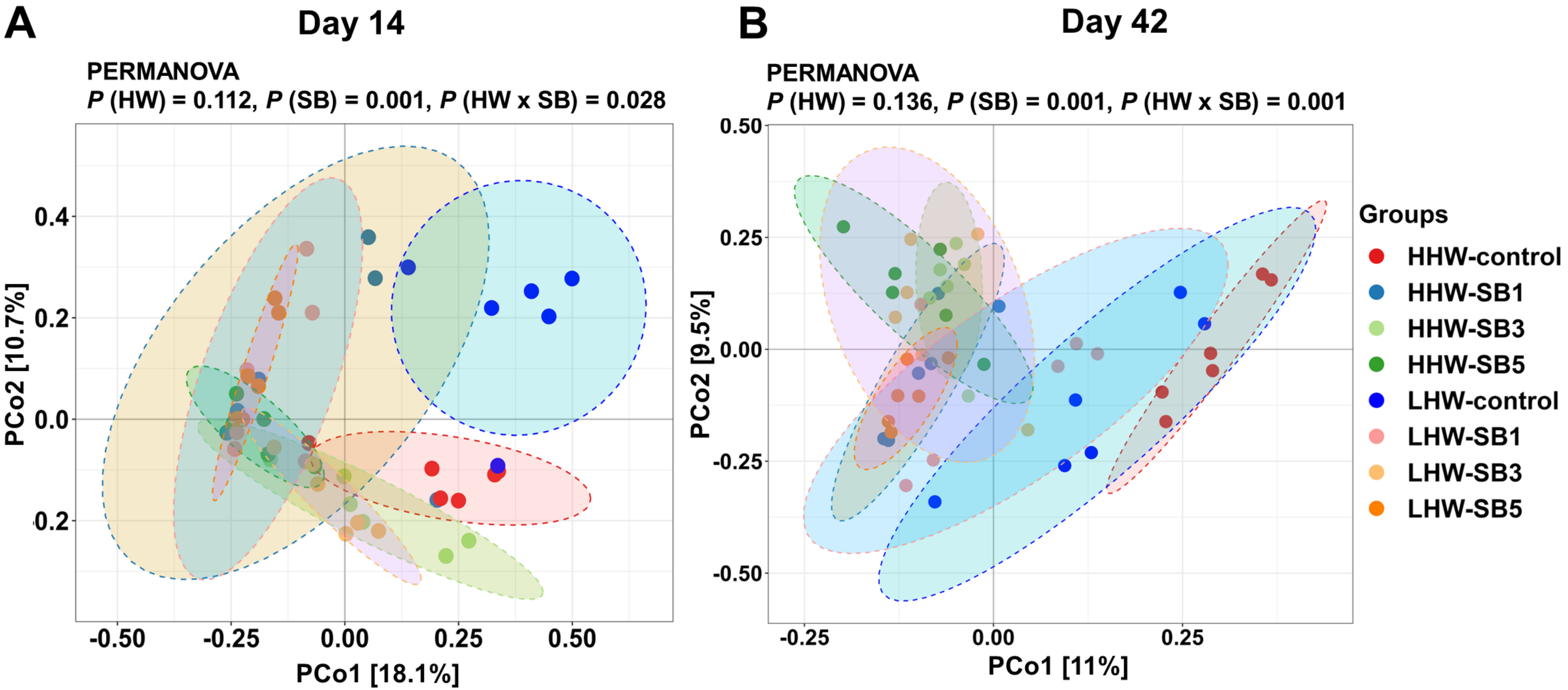
# 4. Caecal microbiota beta diversity



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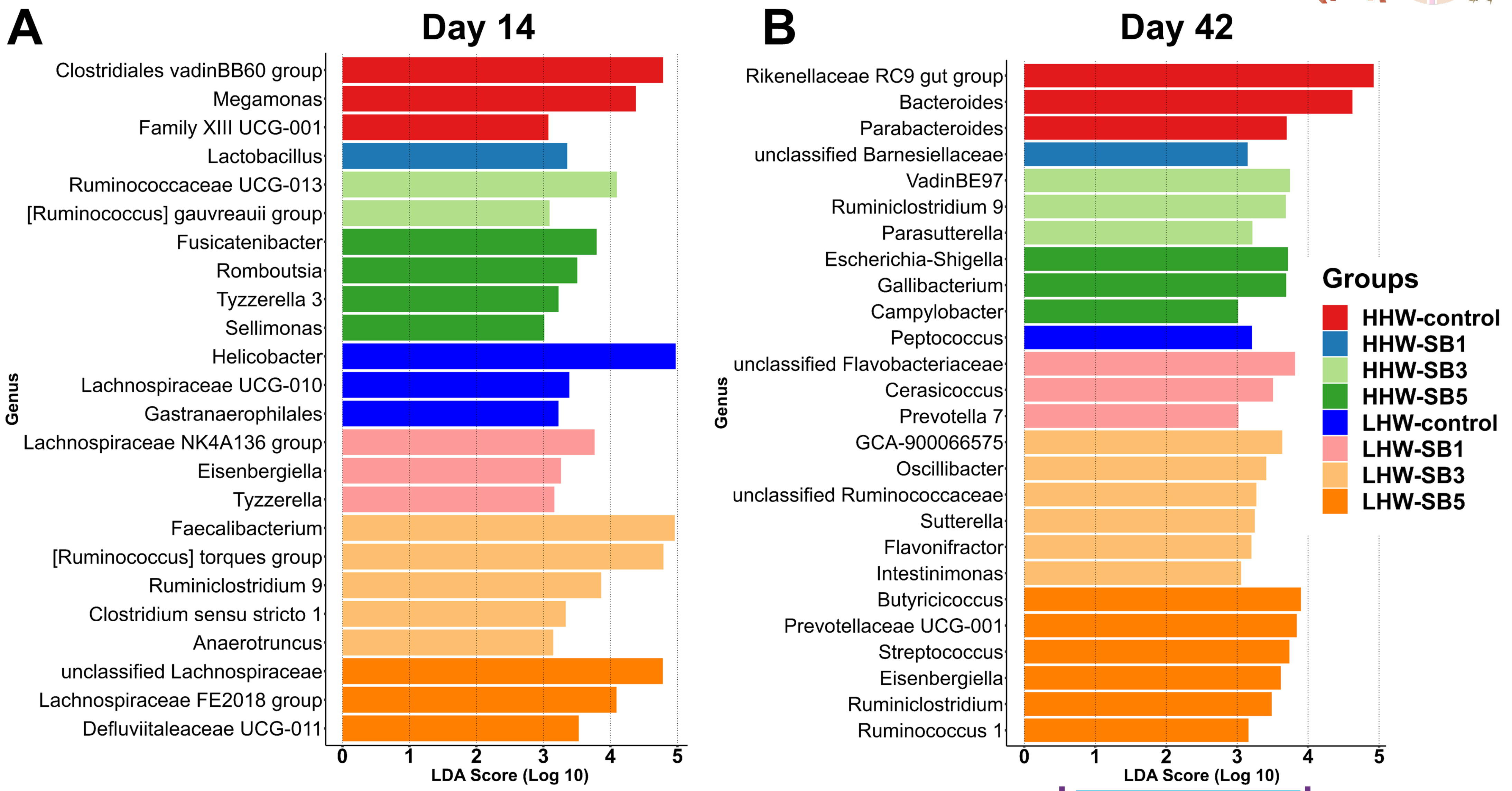
# 4. Differential abundance of bacterial genera



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# Conclusion

- **Hatch-weight** is an important factor influencing subsequent growth performance.
- Effects of SB were **dose-dependent**, with 0.3% significantly enhancing growth, gut barrier function and anti-inflammatory gene expression, and beneficial caecal microbiota.
- In ovo SB injection **enhances growth** in both HW categories, with **strong effects in LHW at 0.3% inclusion**.
- The findings offer **targeted intervention** for improving the growth performance of LHW chicks, leading to more uniform flocks and better production efficiency.



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Optimal gut function in monogastric livestock

03

# Study 3: Effects of coarse corn, oat hulls and their combination on growth performance, intestinal functions and microbiota of underperforming chickens



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# Selection of coarse corn and oat hulls?

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- Chickens may have a requirement for **dietary physical structure** to enhance gut development and health.
- A **well-developed gizzard and GIT** have been shown to improve nutrient digestibility and growth performance of chickens

Coarse corn (CC)
+
Oat hulls (OH)

- Gizzard-stimulatory effect
- Longer digesta retention time
- ↑ digestion and utilization of nutrients
- Modulate gut microbiota

Kheravii et al., 2017; Li et al., 2016; Downs et al., 2023

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# Goal of the study

- To determine the effects of **coarse corn and oat hulls and their combination** on growth performance, GIT development and microbiota of underperforming chickens.

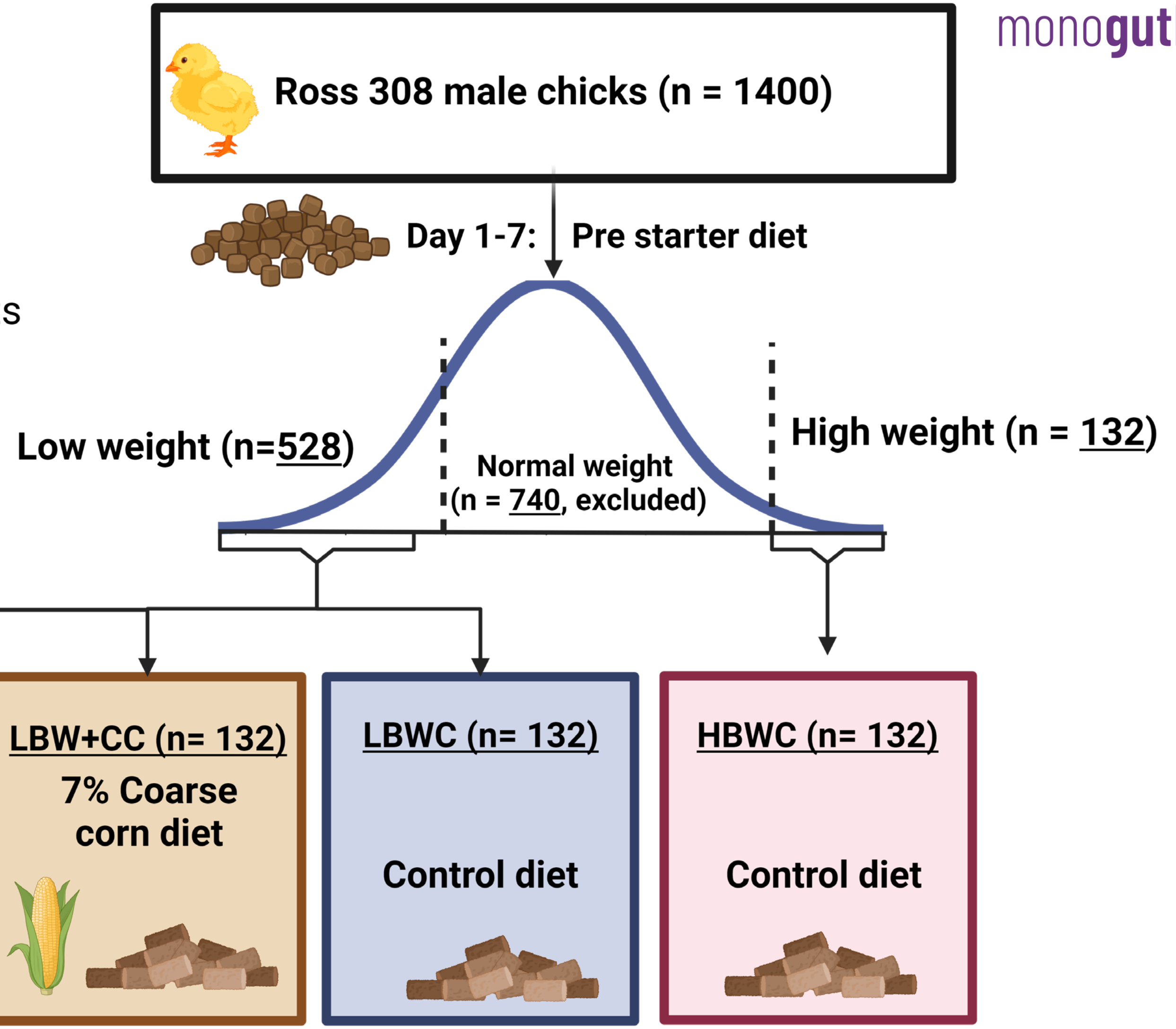
- To evaluate whether **low-weight broiler chickens**, under these dietary treatment, can catch up with their high bodyweight counterparts.



This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Sklodowska-Curie grant agreement No 955374.

# Trial design

**For each group**
- Replicate pens (n=6)
- Chicks (n=22) in each pen
- Iso-caloric and iso-nitrogenous diets



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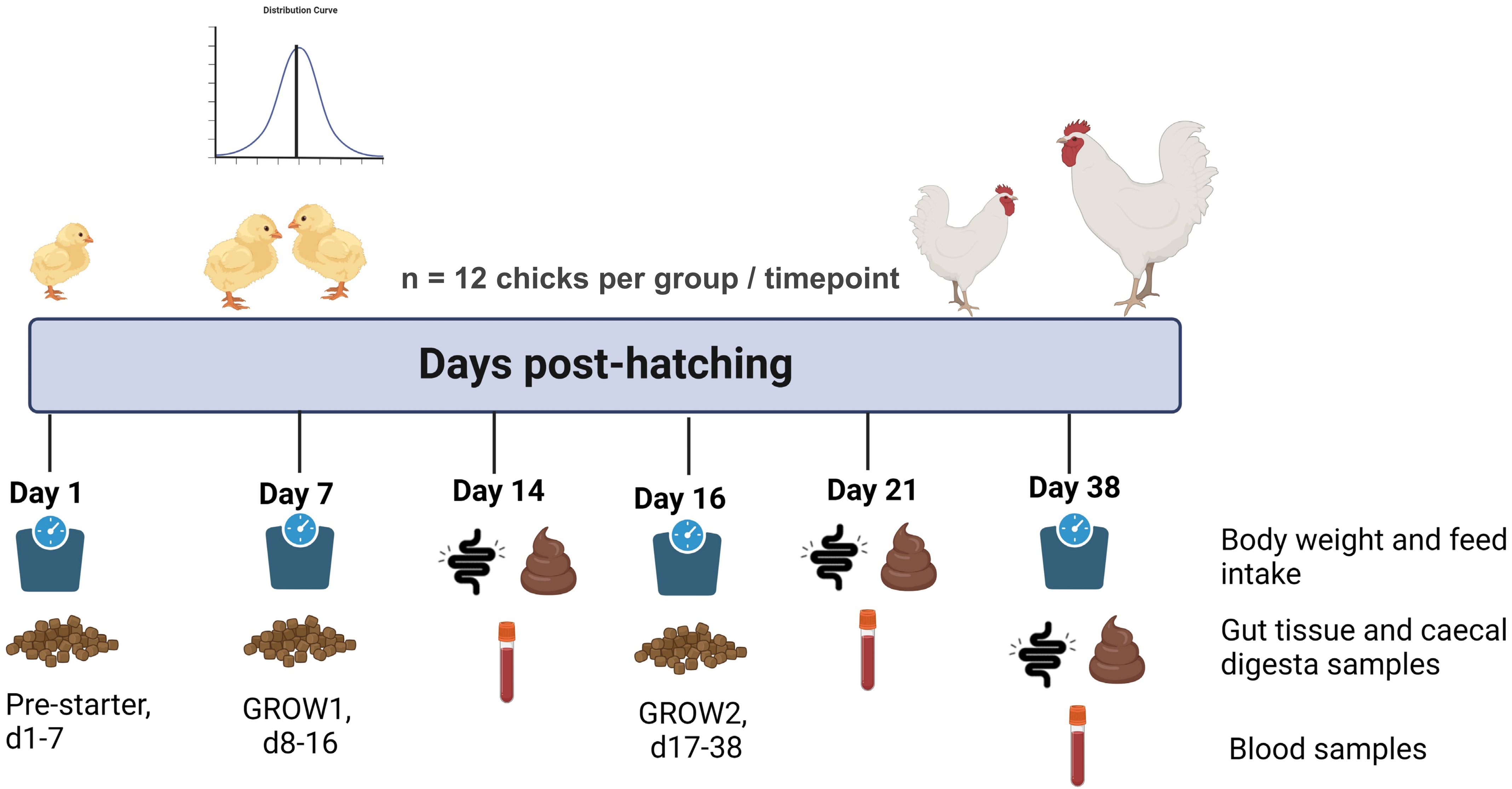
# Experimental timeline



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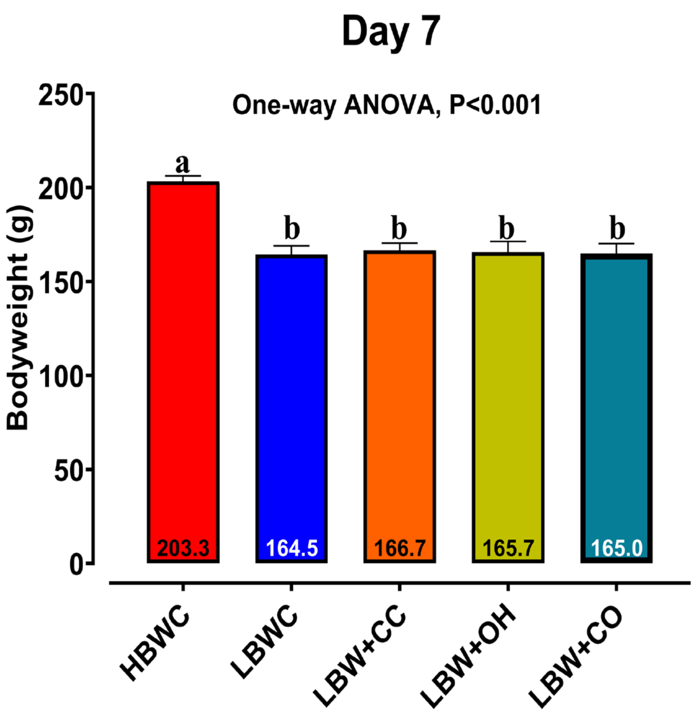
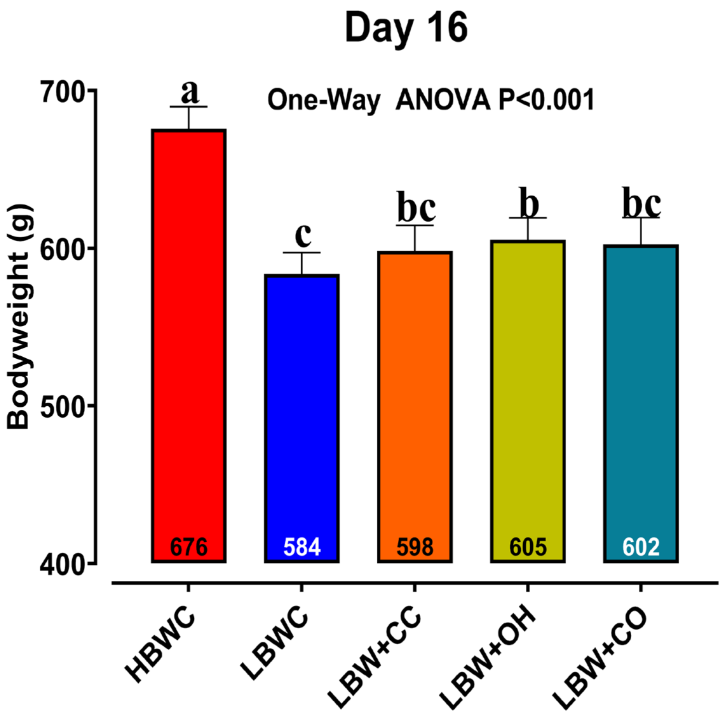
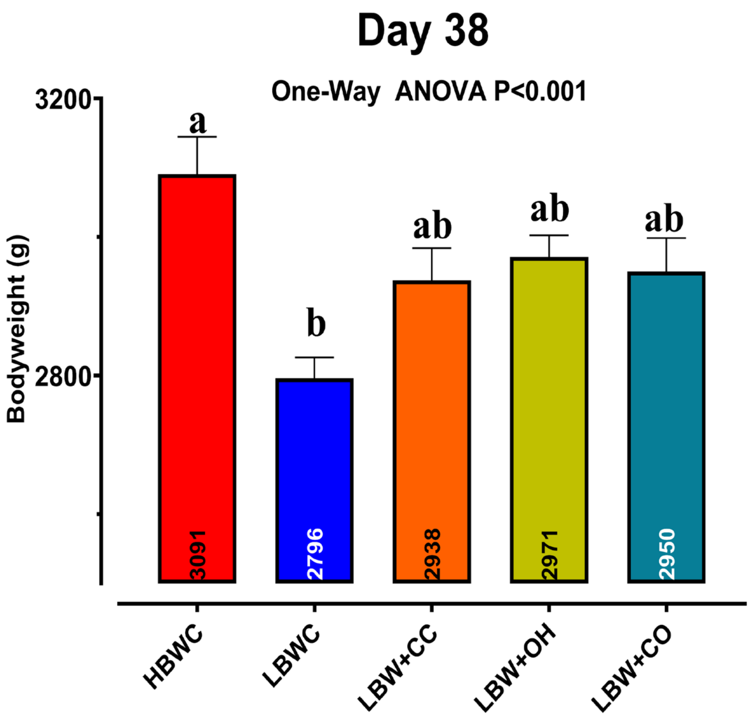
# Body weight



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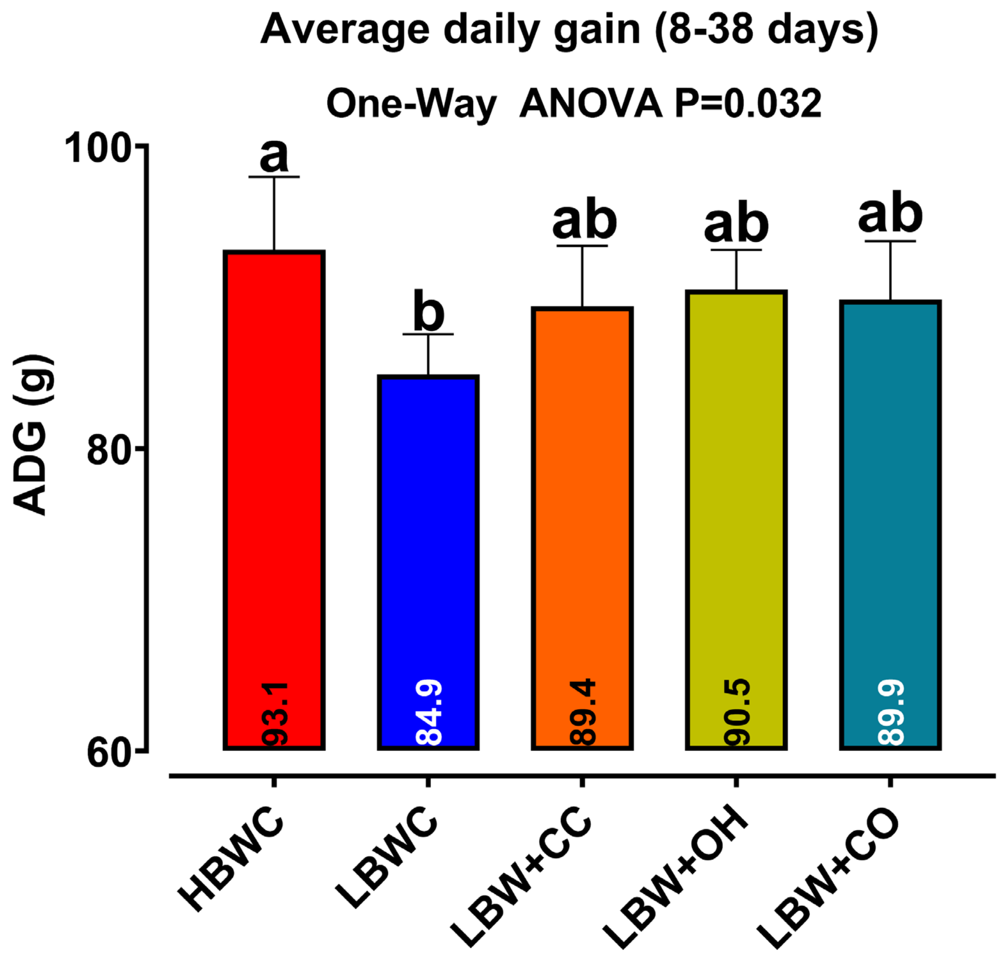
# Average daily gain



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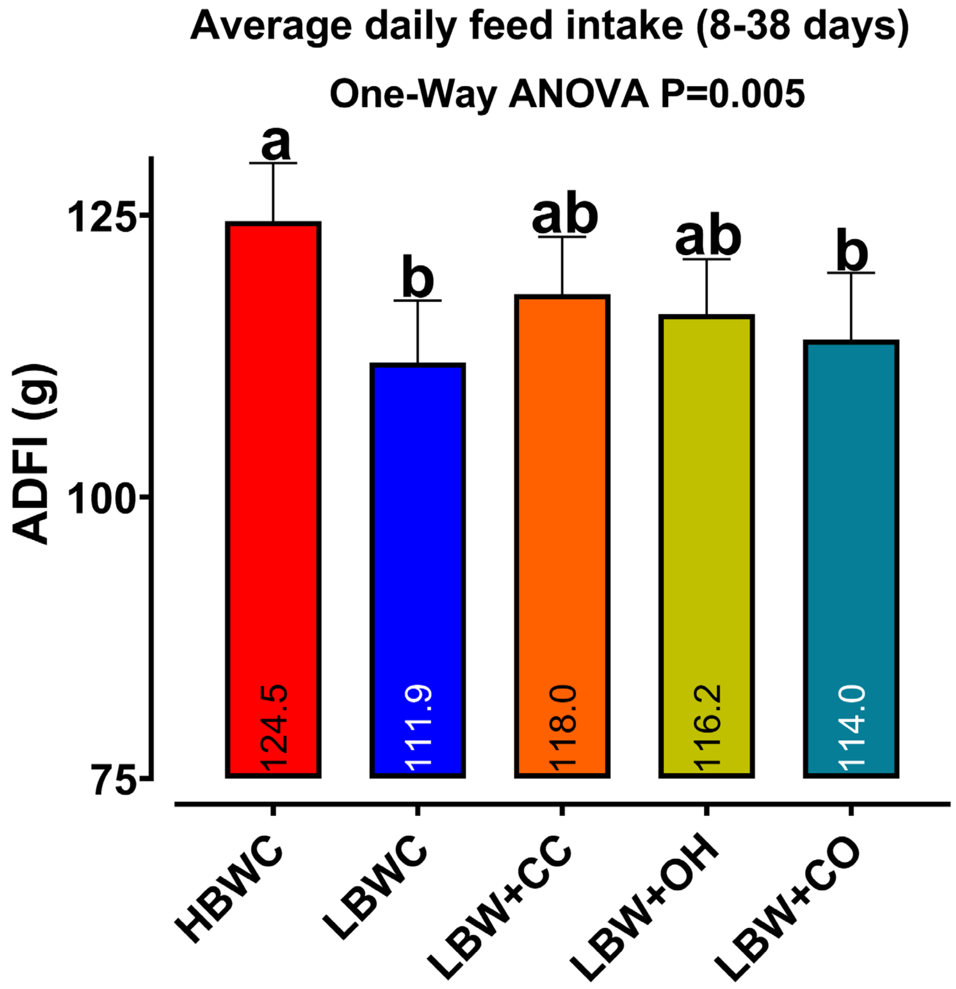
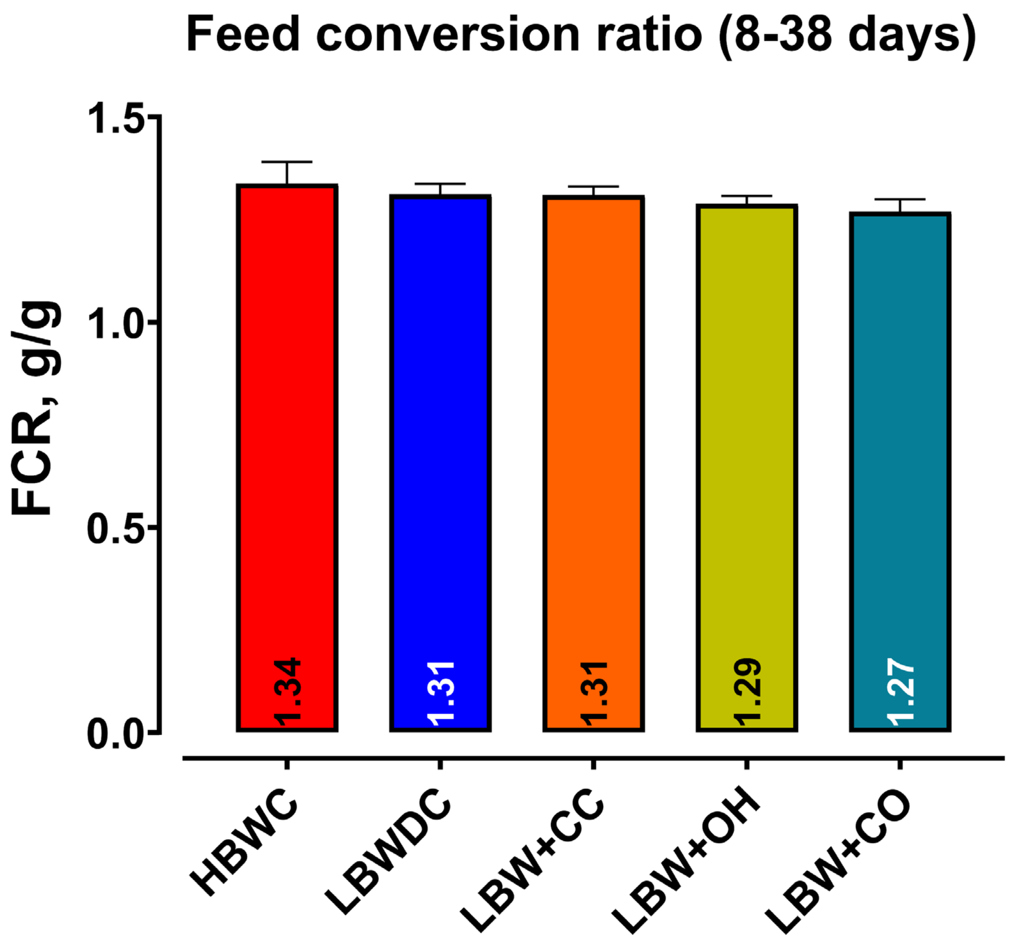
# Feed intake and feed conversion ratio



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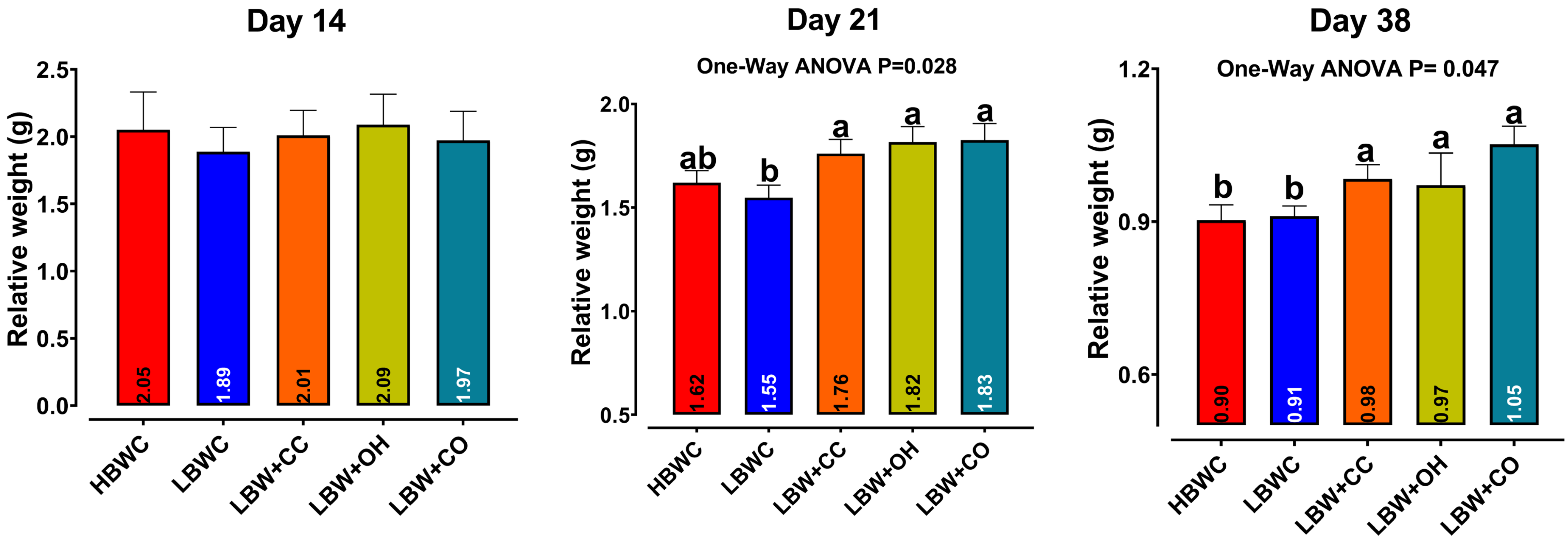
# Gizzard development



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# Conclusion thus far

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- Coarse corn, oat hulls, and their combinations positively **impact GIT development and enhance the Gizzard size** in LBW chickens.
- The LBW groups on experimental diets showed numerically **better performance parameters** compared to LBWC group, paralleling the performance levels of HBWC group.

< Programme

Wednesday 4 September 2024 – Afternoon, 14:00

## Session 98. Nutrition in health and welfare in monogastric animals

Room: Sarda – Palazzo Affari Below ground Floor
Chair: Trocino / Khiaosa-ard
Session Type: Theme session

**14:45 Boosting underperforming broilers: feed structure strategies for better gut development and health, and microbiota**
*M. Z. Akram, E. A. Sureda, N. Everaert*



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# Take home message

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- **Gut Microbiota and transcriptomic related factors** cause divergence in growth rates of broilers within a flock.
- In ovo sodium butyrate administration **differentially affects** broiler chickens with different hatch-weights.
- Optimal dose of 0.3% sodium butyrate significantly affects low hatch-weight chickens, suggesting a **targeted strategy to reduce bodyweight variation** among chickens in a flock.
- **Coarse corn and oat hulls** aided in improving gut development and growth performance of low-weight broilers, paralleling to performance of high-weight broilers – suggest a **potential dietary strategy for improving flock uniformity.**



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# THANK YOU

## Do you have any questions?

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