



Gut microbiota of growing rabbits fed diets with different fiber and lipid contents

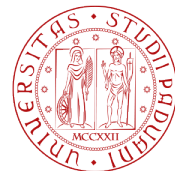
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Introduction

Microbial populations that inhabit animals' gastrointestinal tract constitute their gut microbiota: a complex ecosystem with a very important role in metabolic, nutritional, physiological, and immunological processes.

Animal genetics

Breeding conditions

Environmental status



Nutritional strategies

➤ Starch

➤ Fiber

➤ Protein

➤ Fat

NDF
(Neutral detergent fibre)

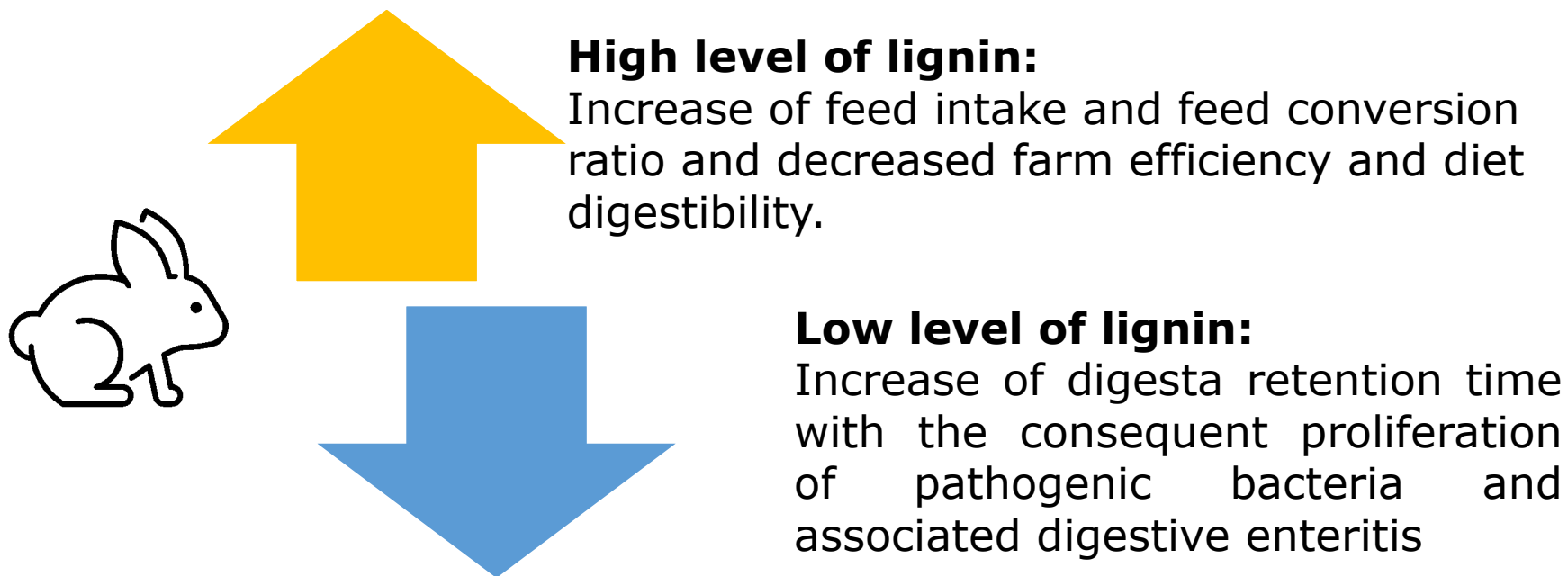
ADF
(Acid detergent fibre)

ADL
(Acid detergent lignin)

Introduction



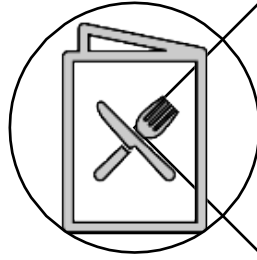
The **ADL fraction**, has been recognized as useful for the prevention of digestive disorders, as it controls the digesta retention time in the gut, affecting the caecal microbial composition, and the corresponding fermentative pattern.



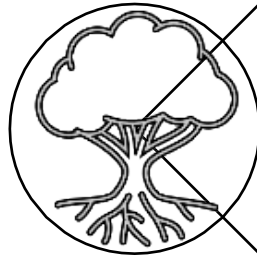
Introduction



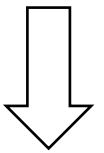
Feeding strategy →



Diet with high ADL and high EE to maintain the energy level



Use of Purified Lignin



Interference with microbial degradation and fermentation of fiber polysaccharides. (Baurhoo et al., 2007)



Objective



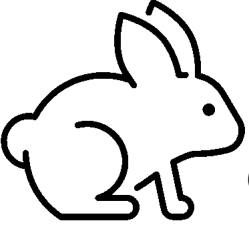
The present study aimed to test the effect of isoenergetic diets including or not purified lignin on gut microbiota composition (in caecal content and hard feces) in growing rabbits.

The study also evaluated the effects on growth performance, diet digestibility, and caecal fermentation.

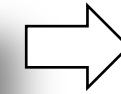


Materials and Methods

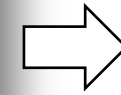
Experimental farm "L. Toniolo" of the University of Padova

576 x 

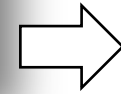
Males and Females crossbred rabbits (Hycole, SARL Hycole, Marcoing, France)



18 elevated pens (210 cm x 92 cm)

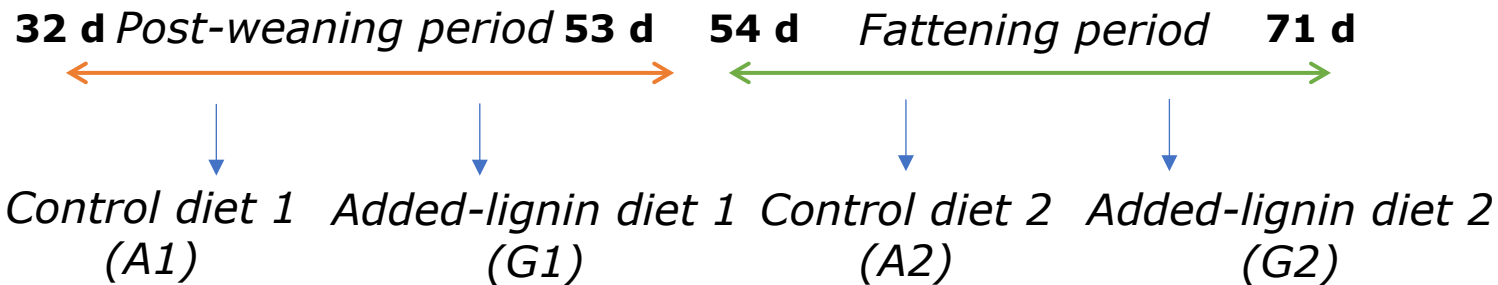


8 automatic nipple drinkers



4 feeders for the manual distribution

Over the time



Diets were formulated consistent with the post-weaning and fattening requirements of rabbits (De Blas and Mateos, 2020; Gidenne et al., 2020)



| Experimental diets | A1 | G1 | A2 | G2 |
|--|-----------|-----------|-----------|-----------|
| Period of administration | 33-54 d | 33-54 d | 55-72 d | 55-72 d |
| Ingredients | | | | |
| Alfalfa meal (CP: 16.5%), g/kg | 200 | 180 | 200 | 180 |
| Alfalfa meal (CP: 14.3%), g/kg | 90.0 | - | - | - |
| Arbocel®, g/kg | - | 50.0 | - | 20.0 |
| Wheat bran + cruschello (CP: 14.5%), g/kg | 225.5 | 224.5 | 229 | 228.5 |
| Barley (CP: 10.26%), g/kg | 150 | 150 | 230 | 200 |
| Dried beet pulp (CP: 8.0%), g/kg | 140 | 120 | 140 | 120 |
| Sunflower meal (CP: 30.0%), g/kg | 150 | 210 | 160 | 190 |
| Soybean oil, g/kg | 10.0 | 30.0 | 10.0 | 30.0 |
| Molasses (50% cane+50% beet), g/kg | 15.0 | 15.0 | 15.0 | 15.0 |
| Calcium carbonate, g/kg | 6.0 | 10.0 | 4.0 | 6.0 |
| Dicalcium phosphate, g/kg | 3.0 | - | 1.5 | - |
| Sodium chloride, g/kg | 4.0 | 4.0 | 40 | 4.0 |
| L-lysine base (77%), g/kg | 1.5 | 1.5 | 1.5 | 1.5 |
| DL-methionine, g/kg | 1.0 | 1.0 | 1.0 | 1.0 |
| Vitamin-mineral premix¹. % | 4.0 | 4.0 | 4.0 | 4.0 |



Materials and Methods

Chemical

composition

| | <u>A1</u> | <u>G1</u> | <u>A2</u> | <u>G2</u> |
|---------------------|-----------|-----------|-----------|-----------|
| Dry matter, g/kg | 909 | 914 | 901 | 906 |
| Crude protein, g/kg | 159 | 156 | 152 | 158 |
| Ether extract, g/kg | 29.3 | 40.0 | 28.8 | 37.8 |
| Crude fiber, g/kg | 146 | 162 | 147 | 154 |
| Starch, g/kg | 101 | 117 | 143 | 138 |
| NDF, g/kg | 345 | 354 | 326 | 331 |
| ADF, g/kg | 189 | 200 | 174 | 177 |
| ADL, g/kg | 48.3 | 53.7 | 42.6 | 47.4 |
| Gross energy, MJ/kg | 16.8 | 17.3 | 16.7 | 17.0 |



Materials and Methods

Laboratory of DAFNAE (Department of Agronomy, Food, Natural resources, Animals and Environment) University of Padova

At 70d, 20 rabbits were slaughtered and 300 mg of hard feces and cecal content was collected in a Falcon tube and stored at -80°C .

1. DNA extraction:

Samples were homogenized at 30 Hz for 6 minutes with TissueLyser (Qiagen).

Enzymatic treatment (Lysozyme/ 30 min at 37°C)

Enzymatic treatment (Proteinase K/ 1h at 50°C)

Samples were incubated at 75°C for 5 min and then centrifuged for 5 min at $20.000 \times g$, after which the supernatant was collected

2. Purification:

Automated DNA extraction - Biosprint 96:

The sample is subjected to various washing cycles with different solutions to purify the DNA from any inhibitors





Materials and Methods

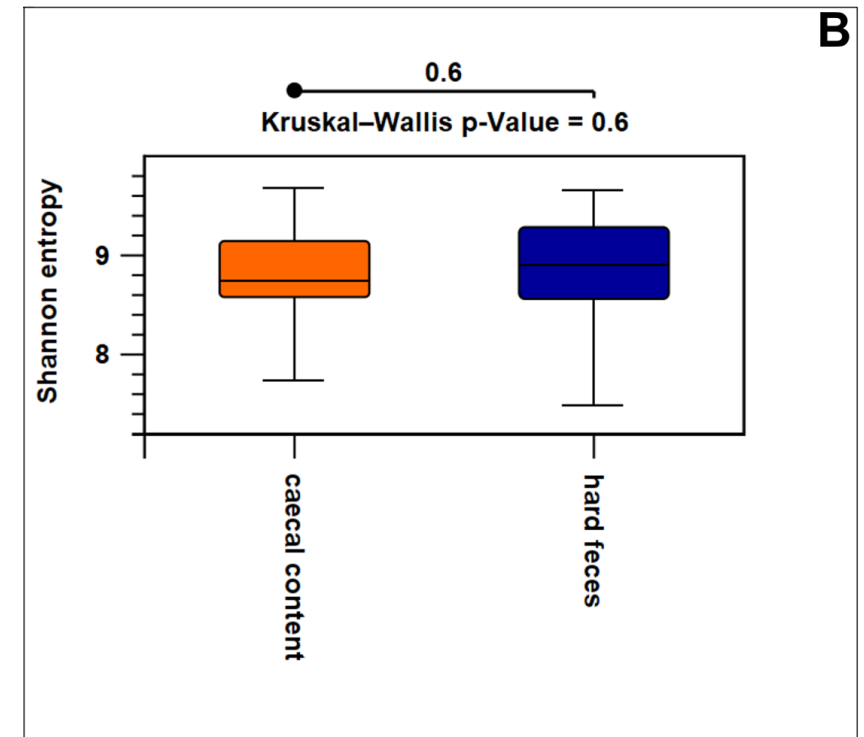
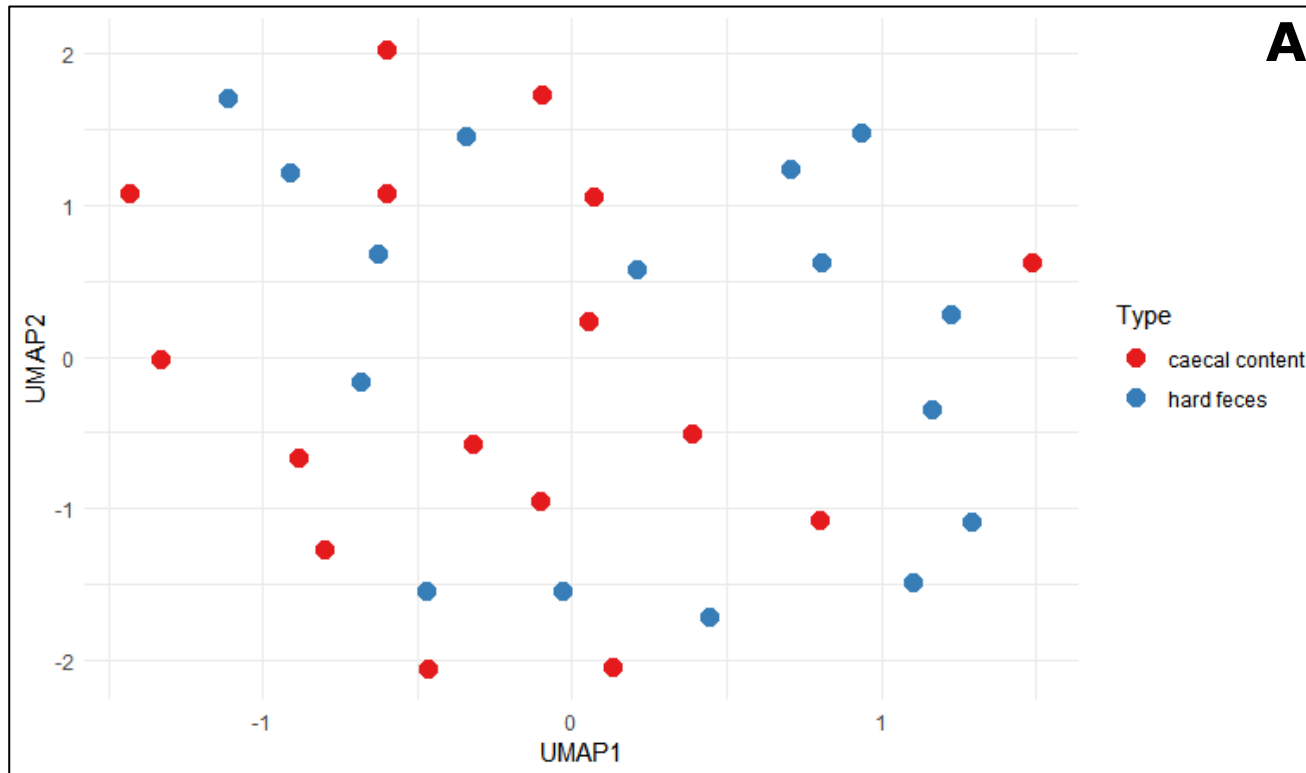
Laboratory of DAFNAE (Department of Agronomy, Food, Natural resources, Animals and Environment) University of Padova

- **Metabarcoding of bacterial 16S rRNA gene by next-generation sequencing (NGS):** Library preparation was carried out using the *16S Ion Metagenomics Kit* (Thermo Fisher Scientific) for the amplification of hypervariable regions (V2, V4, V8 and V3, V6-7, V9).
- Then, the protocol involved the use of the *Ion Xpress Plus 9 Fragment Library Kit* (Thermo Fisher Scientific) and *Ion Express Barcode Kit* (Thermo Fisher Scientific) for barcode ligation. Sequencing was done with Ion GeneStudio S5 using the Ion 520 chip kit (Thermo Fisher Scientific).
- The data were analyzed using the *CLC Genomic Workbench 22.0 (Qiagen)* software and **Silva SSU 99%** database for the OTU clustering.





Results: *hard feces vs. caecal content*

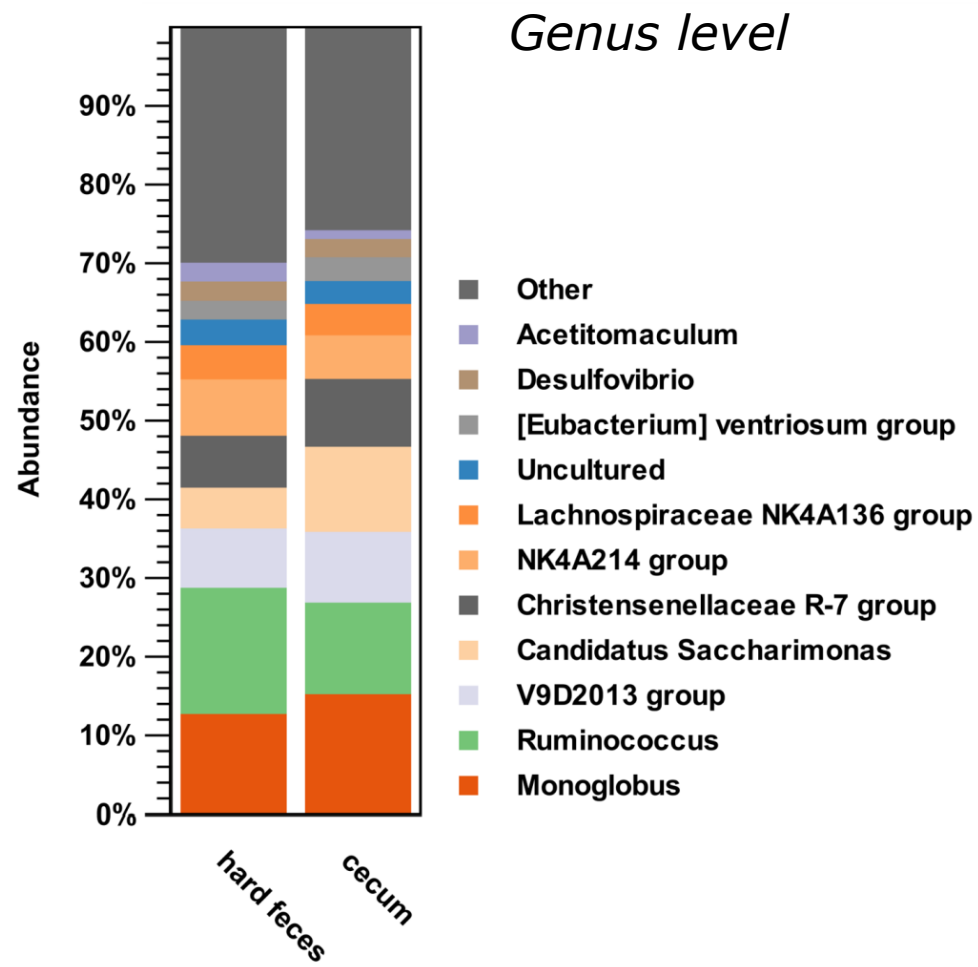
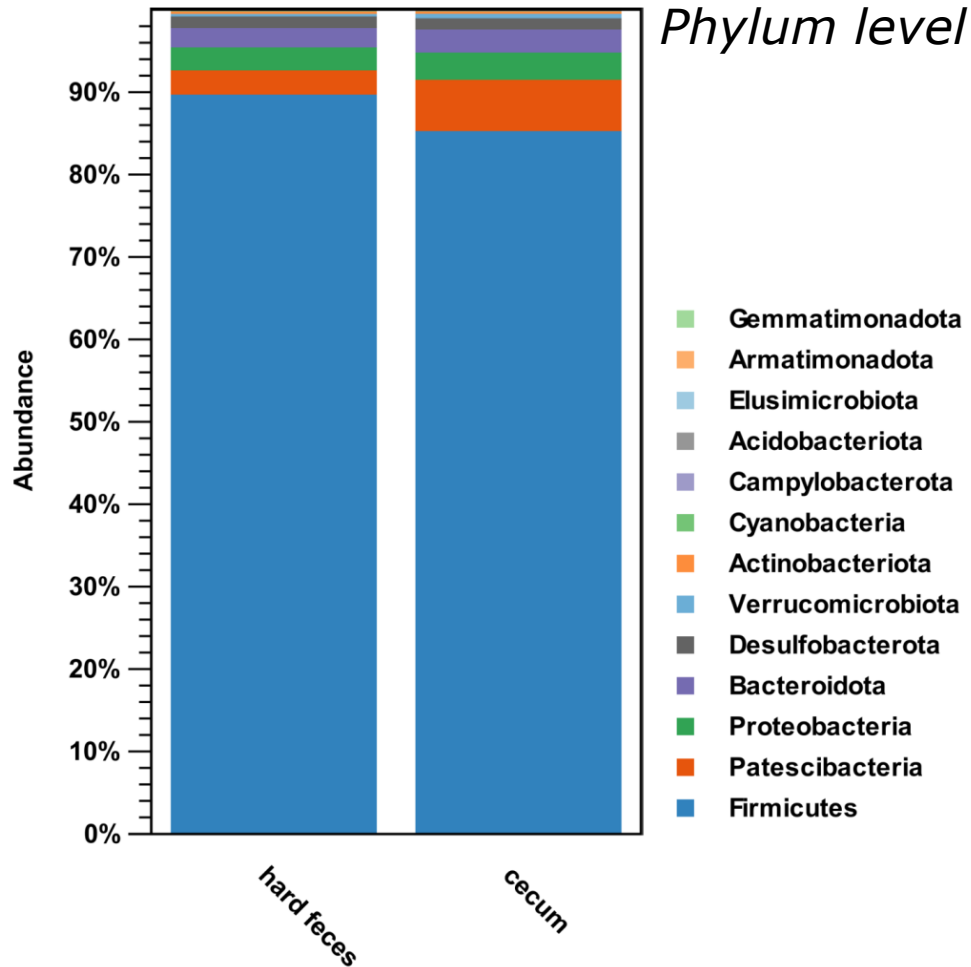


A: *Beta-diversity (Uniform Manifold Approximation and Projection) at OTUs level at 72 days of age*

B: *Alpha-diversity (Shannon index) at OTUs level at 72 days of age*

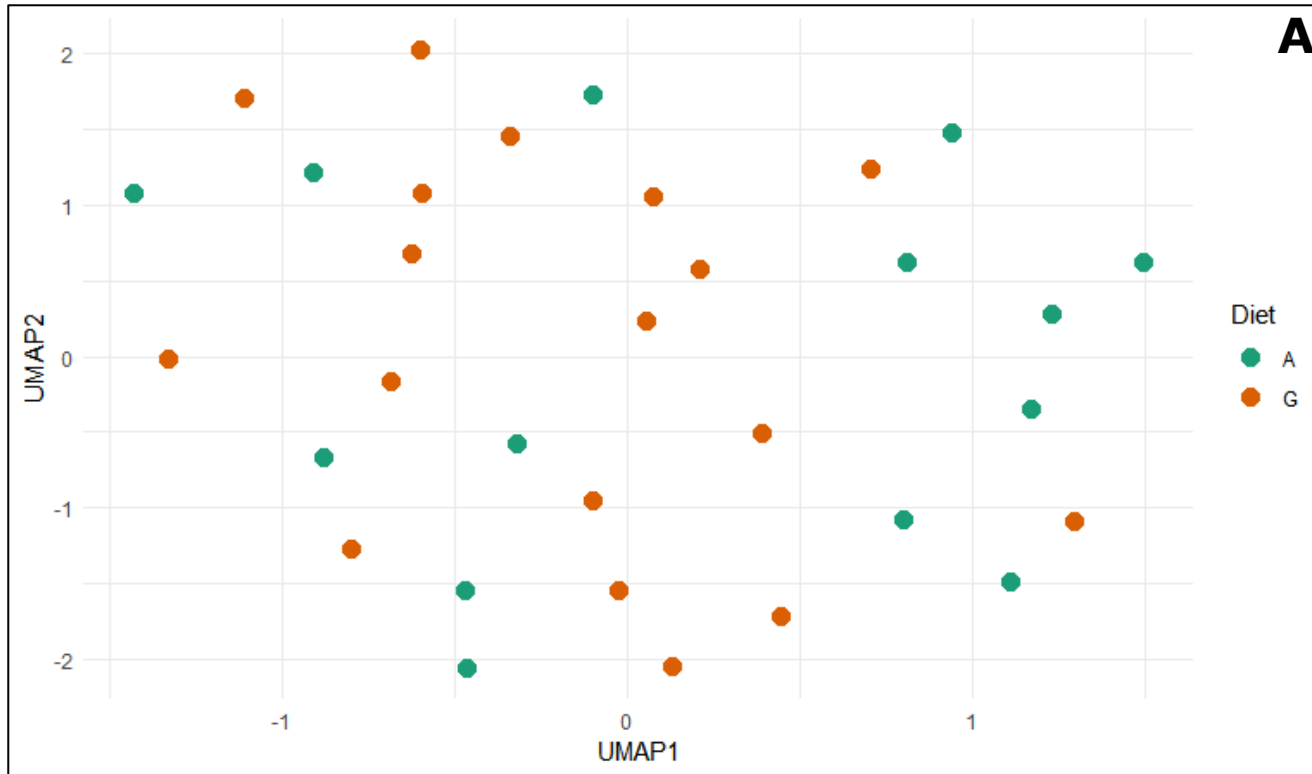


Results: *hard feces vs. caecal content*

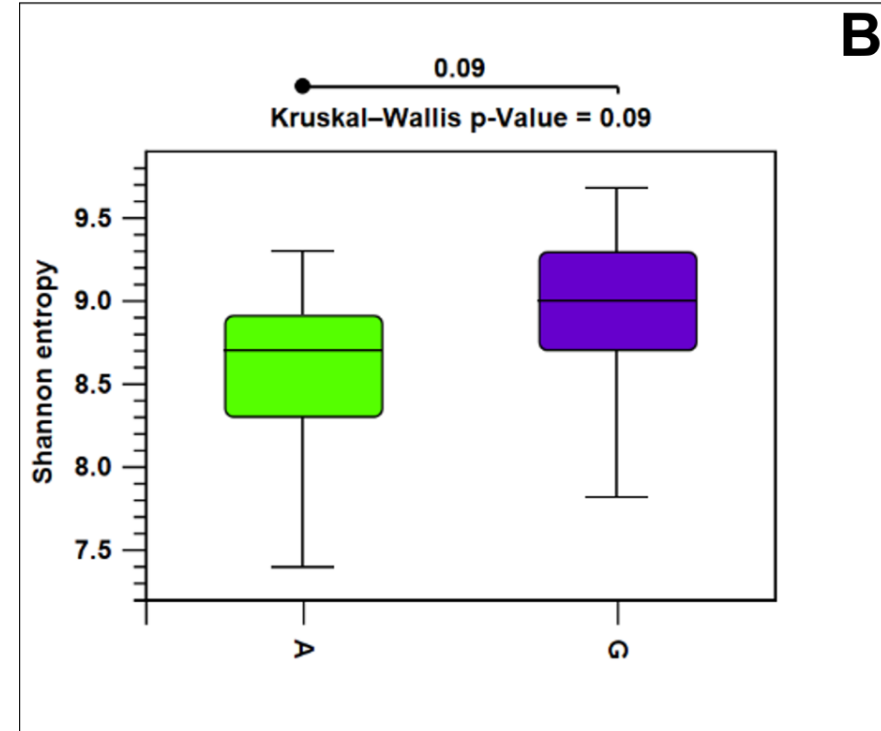




Results: *added-lignin diet vs. control diet*



A: *Beta-diversity (Uniform Manifold Approximation and Projection) at OTUs level at 72 days of age*



B: *Alpha-diversity (Shannon index) at OTUs level at 72 days of age*

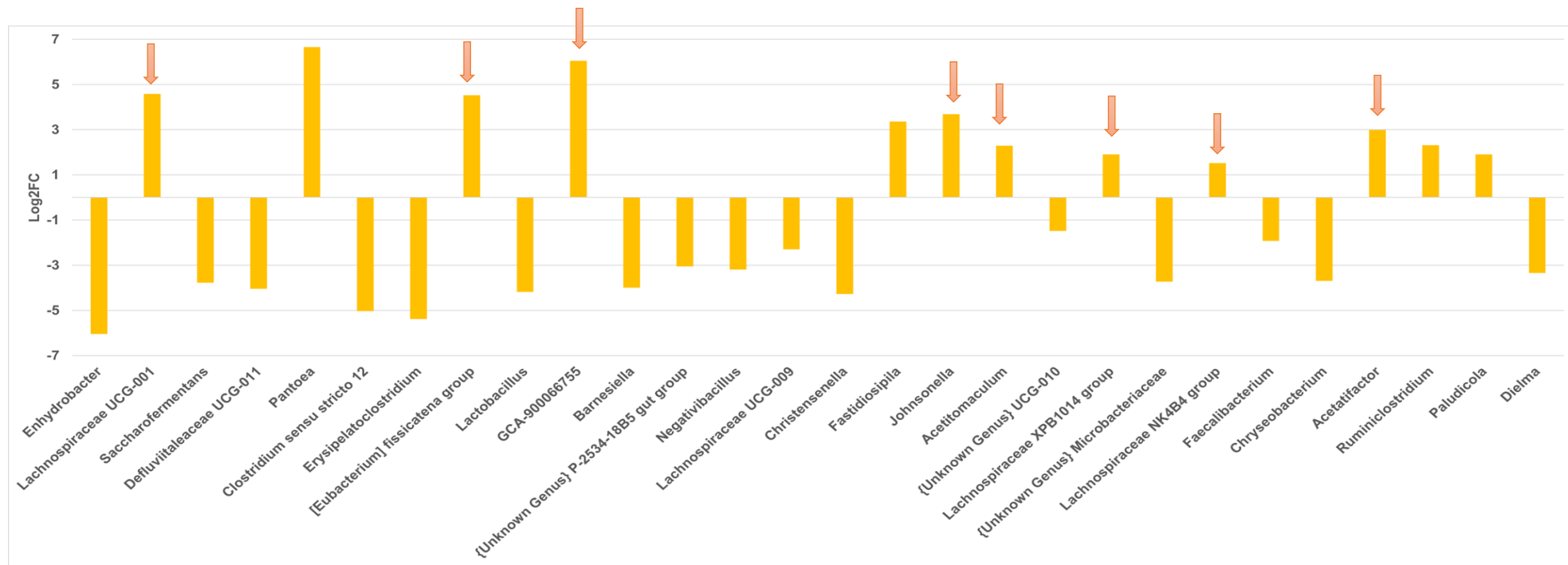


Results: added-lignin diet vs. control diet

Clustered with the Log₂ fold bar chart change values of significantly different (Wald test. $p\text{-value} \leq 0.05$) genera between G and A diets microbial communities.

→ Genera with positive values are overexpressed in animals fed the G diet.

→ Genera with negative values are overexpressed in animals fed the A control diet.

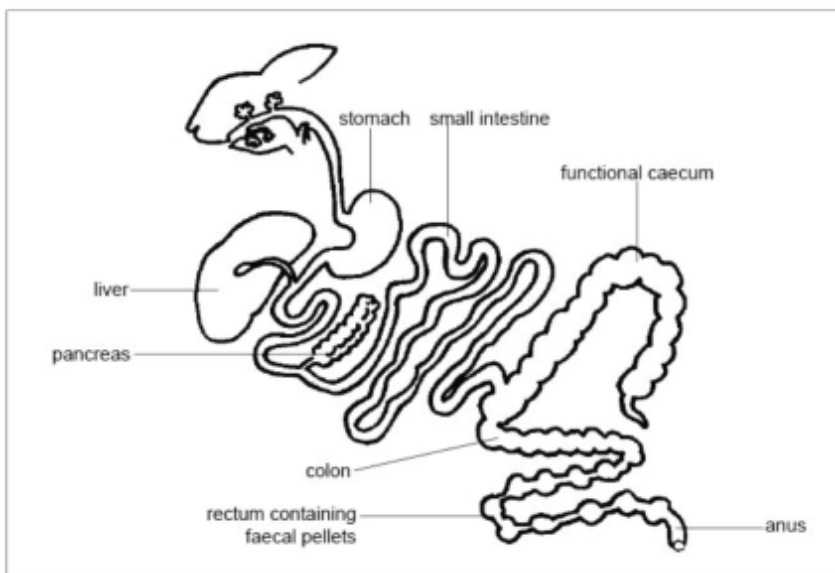




Discussion

1.

HARD FECES = CAECAL CONTENT



Strict proximity between caecum and rectum



Caecotrophy did not occur during the sampling



Feces sampling directly from the anus avoided environmental contamination



Discussion

2.

Dietary inclusion of purified lignin associated with the addition of fat could be a feeding strategy without negative effects on animal gut microbiota.



The higher abundance of genera belonging to the Lachnospiraceae family is reflected in a different fermentative pattern of VFA (volatile fatty acids), but not in the total VFA concentration.



Minor interference with microbial degradation and fermentation of fiber polysaccharides.



Discussion

2.

| | Experimental diets | | p-value | RSD |
|--|--------------------|------------------|---------|------|
| | Control (A) | Lignin-added (G) | | |
| → Total volatile fatty acids (VFA) (mmol/L) | 54.8 | 55.4 | 0.892 | 9.56 |
| → C2 (mmol/100 mmol VFA) | 76.2 | 73.2 | 0.058 | 3.28 |
| → C3 (mmol/100 mmol VFA) | 4.7 | 4.1 | 0.301 | 1.15 |
| → C4 (mmol/100 mmol VFA) | 17.8 | 21.2 | 0.051 | 3.44 |
| C5 (mmol/100 mmol VFA) | 1.1 | 1.2 | 0.455 | 0.29 |
| C6 (mmol/100 mmol VFA) | 0.18 | 0.36 | 0.174 | 0.28 |



Conclusion



Hard feces resulted in a good alternative to studying cecum microbiota without euthanizing.



Purified lignin does not represent a barrier to the digestion of main nutrients coming from the other raw materials.



Further studies should focus on harnessing the metabolic pathways of bacteria by a metagenomic approach for improving nutritional health → *PRECISION BIOTIC APPROACH*



Thanks!

Any questions?

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