UNIVERSITÀ DEGLI STUDI DI MILANO
DIPARTIMENTO DI MEDICINA VETERINARIA
E SCIENZE ANIMALI

Effects of yeast nucleotides administration via drinking water on broiler chickens

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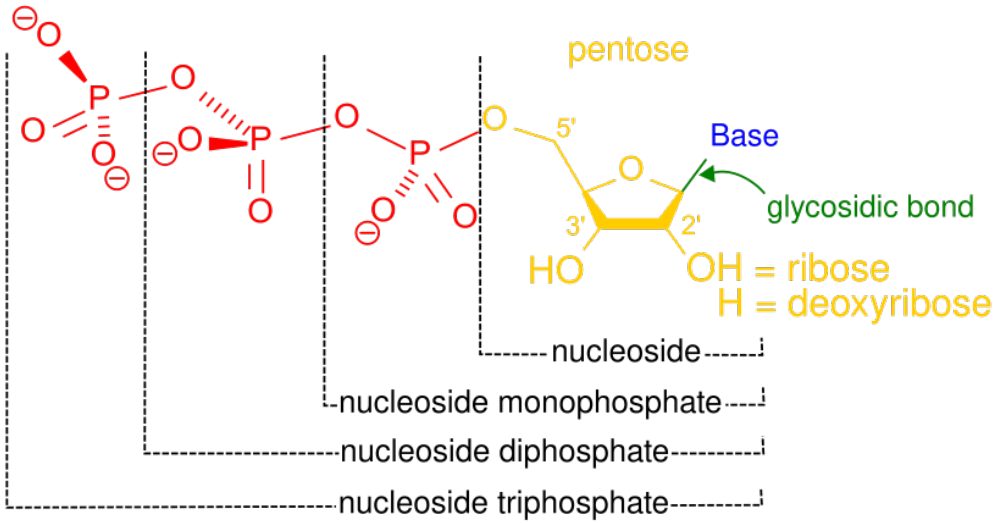
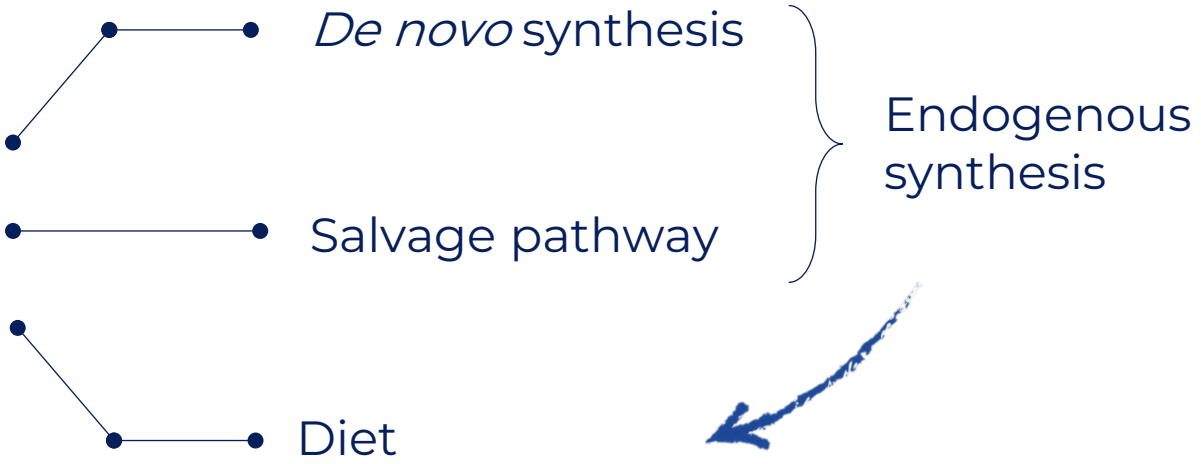
Nucleotides





Numerous functions:

- Building blocks of nucleic acids
- Source of chemical energy (ATP, GTP)
- Participate in cellular signaling
- Cofactors of enzymatic reactions
- Maturation of enterocytes

Conditionally essential nutrients

Periods of rapid growth, disease challenges, low levels of feed consumption

Limited studies on the employment of nucleotides in broiler chickens

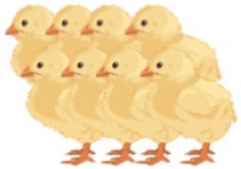
- Variable results on growth performance, intestinal morphology and gene expression
- Yeast-nucleotides were administered only via feed
- Scarce information on the effect on gut microbiota

Aim of the study

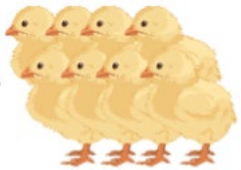
To evaluate the effect of yeast nucleotide administration via drinking water on broiler chickens' growth performance and intestinal health parameters.

Experimental treatments and diet

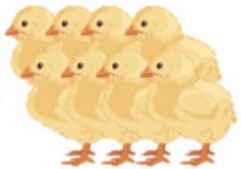
480 one-day-old male broiler chickens



CTR
No supplementation → 8 replicates, 20 chickens/replicate



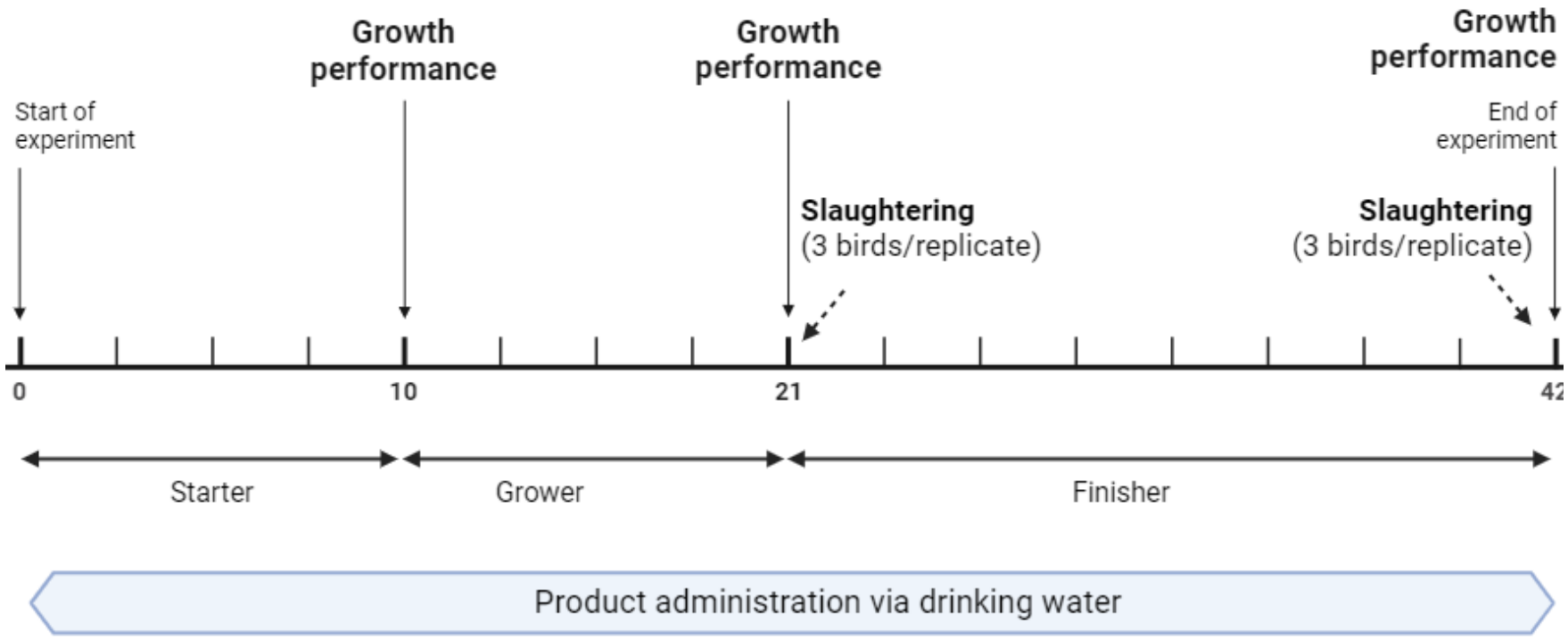
T1
Yeast-nucleotides 90g/ton water → 8 replicates, 20 chickens/replicate



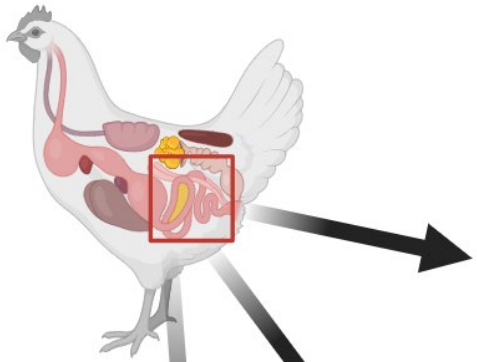
T2
Yeast-nucleotides 180g/ton water → 8 replicates, 20 chickens/replicate

	Starter	Grower	Finisher
Wheat	45.6	45.4	47.3
Soybean meal 48%	34.0	32.0	27.0
Corn	8.0	6.4	7.5
Soybean oil	4.8	6.0	7.2
Barley	3.5	4.5	5.1
Rice hulls	/	2.3	2.8
Calcium carbonate	1.30	1.00	0.91
Dicalcium phosphate	1.30	1.00	0.80
Min-Vit Premix	0.50	0.50	0.50
Salt	0.45	0.45	0.45
Methionine DL	0.26	0.20	0.20
L-Lysine HCL	0.22	0.09	0.13
L-Threonine	0.10	0.05	0.08
Xylanase	0.0050	0.0050	0.0050
Phytase	0.0004	0.0004	0.0004
	45.6	45.4	47.3
Total	34.0	32.0	27.0

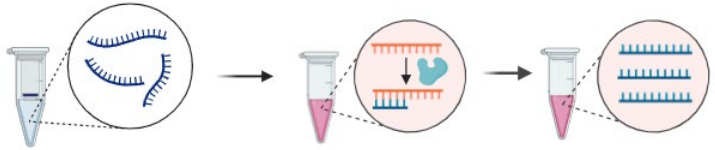
Experimental design



Sample collection and analysis



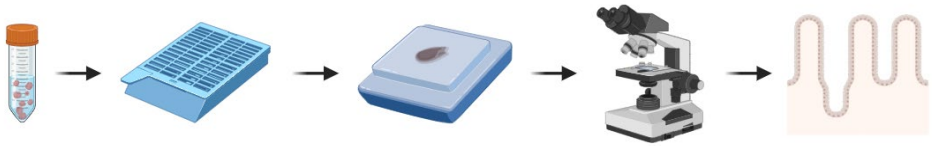
Gene expression
(jejunum mucosa)



mRNA extraction | Reverse transcription | qPCR

- ZO-1, OCLD, CLD3
- MUC2
- Housekeeping genes (GAPDH, RPL4,YWHAZ)

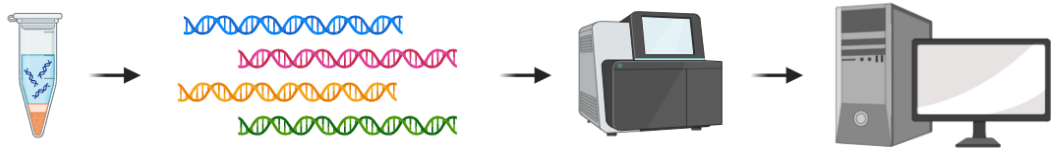
Intestinal morphology
(jejunum tissue)



Paraffin-embedded sample | Intestinal morphology evaluation

- Villus height, crypt depth, V/C ratio
- Mucus thickness

Microbiota
(ceca content)



DNA extraction | Library preparation | Sequencing | Bioinformatics

Statistical analysis

- Growth performance (BW, ADG, ADFI, ADWI, FCR)
- Slaughter performance (carcass weight and yield)
- Intestinal morphology
- Gene expression

Experimental unit

- Pen (growth performance)
- Individual chicken (slaughter performance, gene expression, intestinal morphology, microbiota)

→
- Test for normality (Shapiro-Wilk test)
- 2-way ANOVA, followed by Tukey's multiple comparison tests

Prism

- Microbiota

→
- Alpha diversity indices and Bray-Curtis dissimilarities (beta diversity)
  → Python-based Qiime 1.9 software suite
- Statistical comparisons between treatments (including PERMANOVA)

R

Growth performance

	day	CTR	T1	T2	SEM	p-value
BW (g)	0	46.48	46.19	46.14	0.152	0.6386
	10	298.3	302.8	307.5	2.164	0.2274
	21	**902.9** [b]	924.4	**940.4** [a]	6.030	**0.0311**
	42	2705	2702	2809	20.158	**0.0399**

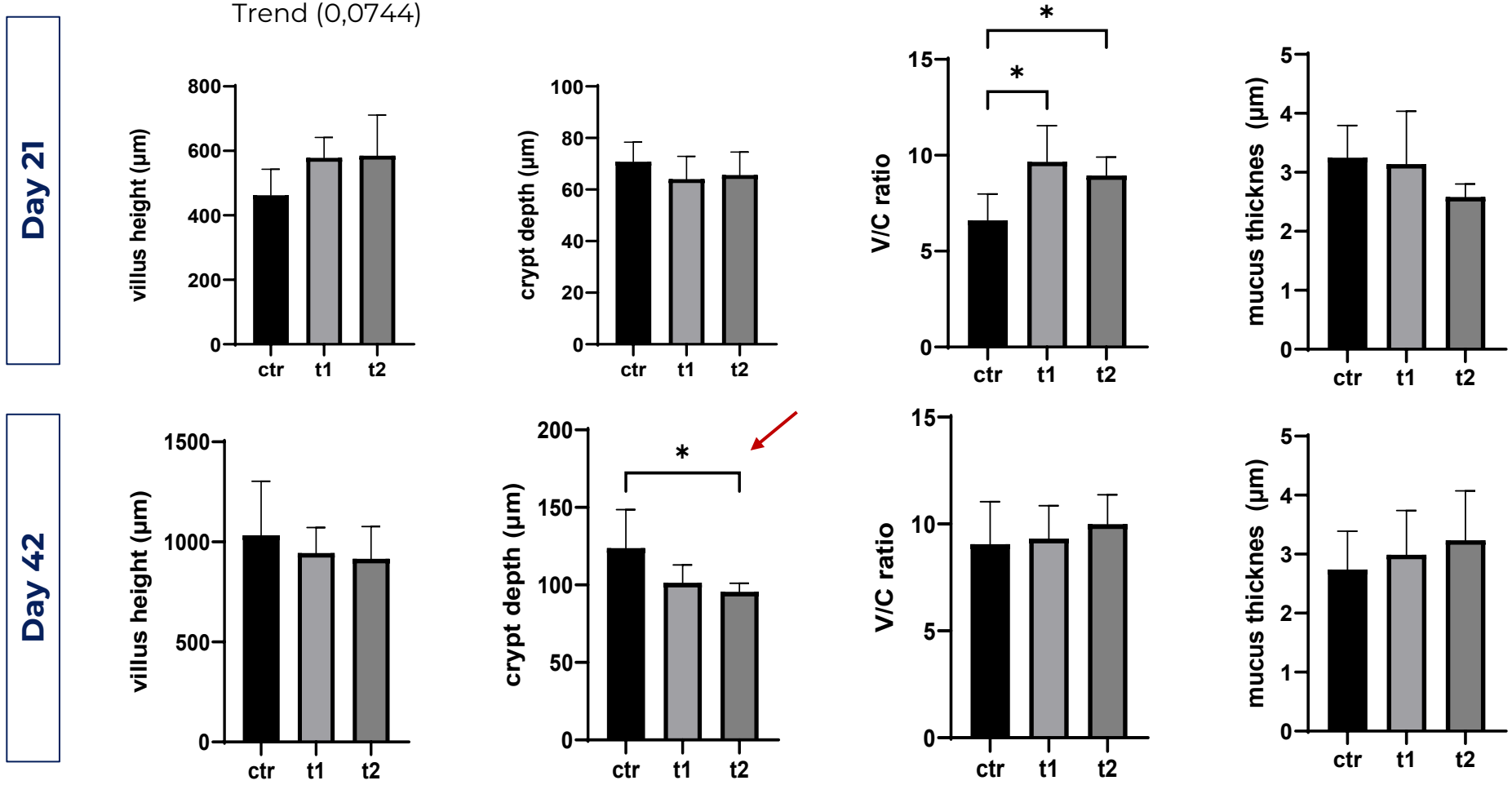
	Phase	day	CTR	T1	T2	SEM	p-value
ADG (g)	Starter	0-10	25.19	25.66	26.09	0.229	0.2796
	Grower	**10-21**	**54.15** [b]	56.3	**57.39** [a]	0.552	**0.0429**
	Finisher	21-42	81.26	78.75	82.55	0.932	0.247
	Overall	0-42	63.3	63.2	65.8	0.480	**0.0393**
ADFI (g)	Starter	0-10	28.89	30.09	29.56	0.265	0.1892
	Grower	10-21	86.11	88.2	89.41	0.838	0.276
	Finisher	21-42	144.4	147.3	148.8	1.438	0.4589
	Overall	0-42	108.1	110.9	112.9	0.996	0.1469
FCR	Starter	0-10	1.148	1.174	1.135	0.007	*0.0702*
	Grower	10-21	1.593	1.568	1.565	0.014	0.7165
	Finisher	21-42	1.776	1.871	1.81	0.021	0.1673
	Overall	0-42	1.75	1.81	1.76	0.015	0.303
ADWI (l)	Starter	0-10	0.064	0.068	0.069	0.002	0.4736
	Grower	10-21	0.196	0.214	0.208	0.004	0.12310
	Finisher	21-42	0.651	0.652	0.359	0.004	0.6478

Slaughtering performance

day	Item	CTR	T1	T2	SEM	p-value
21	Carcass weight (g)	**607.5 b**	621.1	**633.1 a**	**4.081**	**0.0353**
	Carcass yield (%)	66.66	66.68	66.89	0.196	0.8694
	Intestinal weight (g)	60.29	61.01	63.91	1.300	0.3632
42	Carcass weight (g)	1883	1938	1902	13.059	0.2229
	Carcass yield (%)	69.82	69.48	68.71	0.356	0.4332
	Intestinal weight (g)	106.1	111.5	115.8	1.940	0.1246

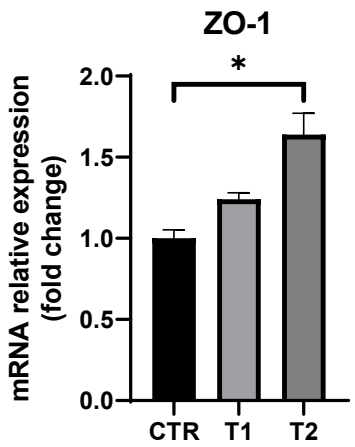
Jejunum morphology

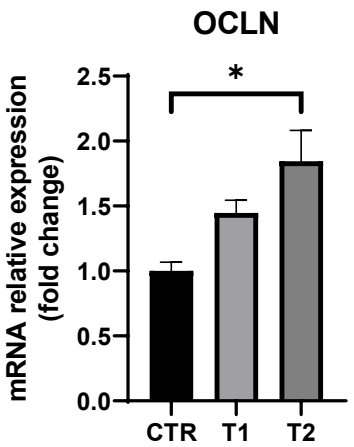
Trend (0,0744)

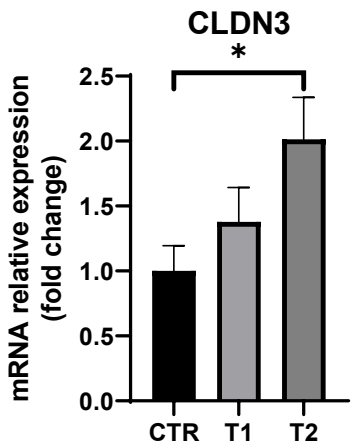


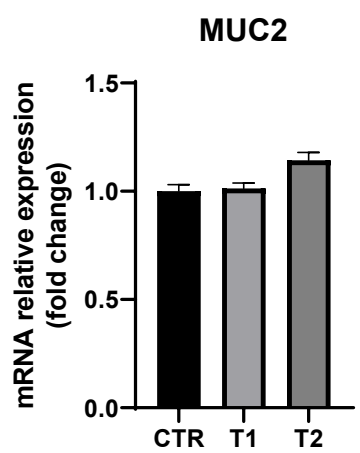
Gene expression

Day 21

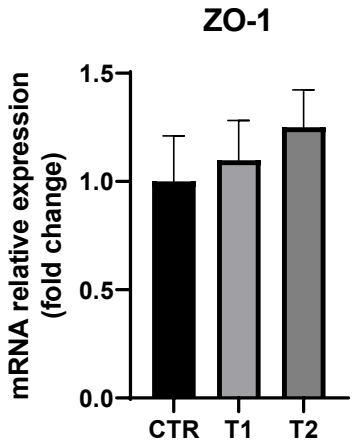


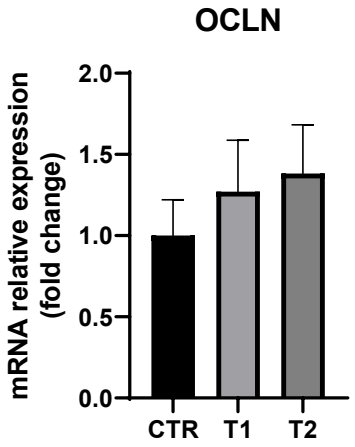


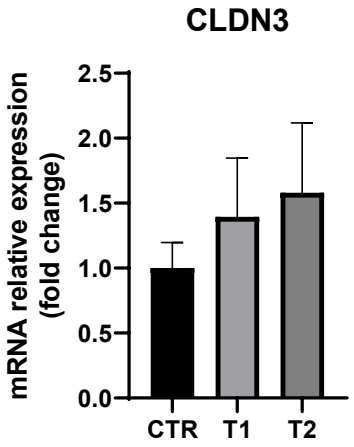


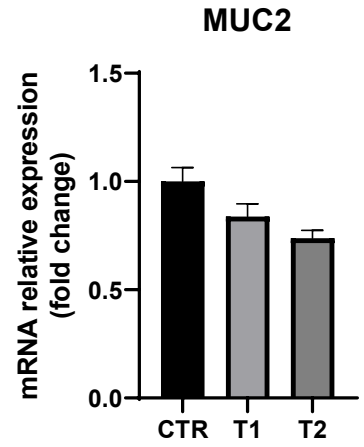


Day 42

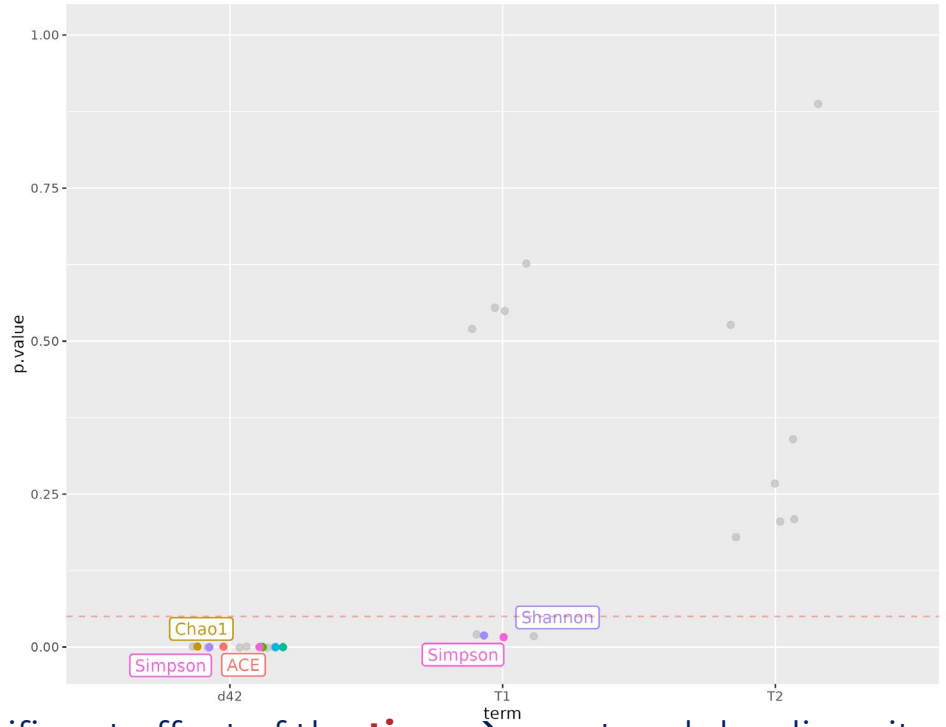




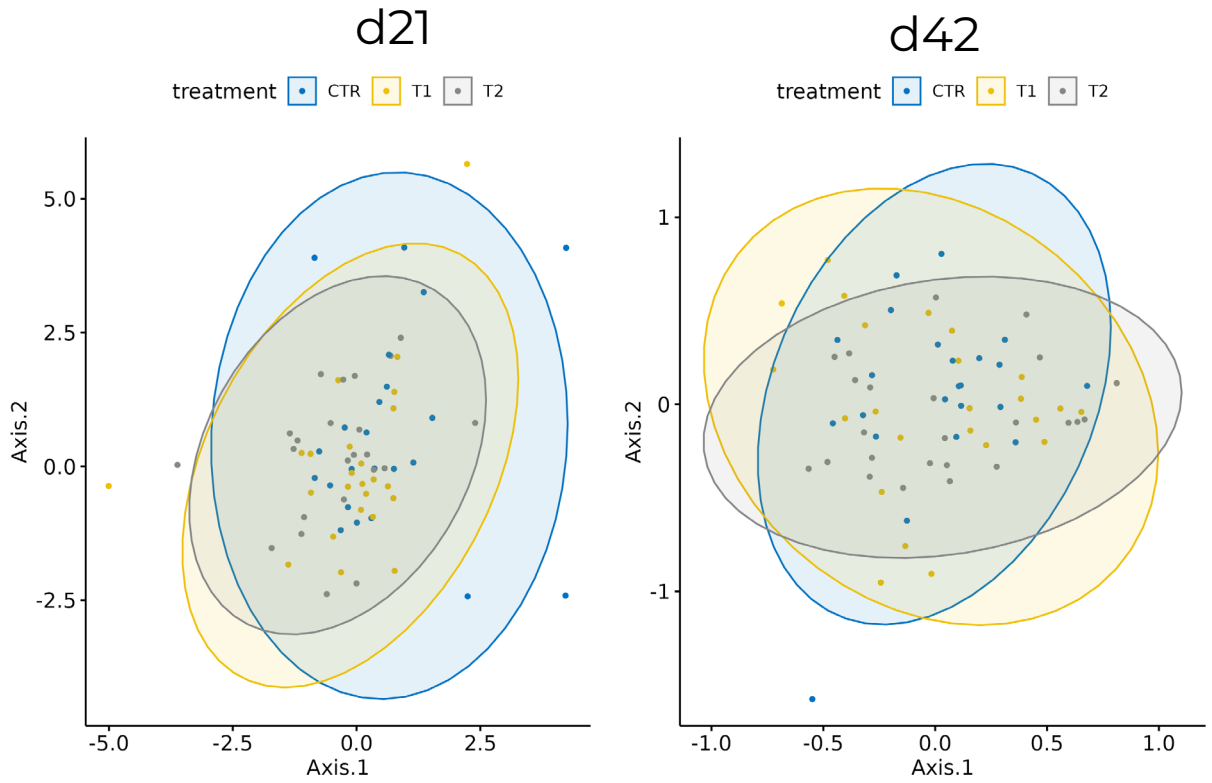




Cecal microbiota – alpha and beta diversity

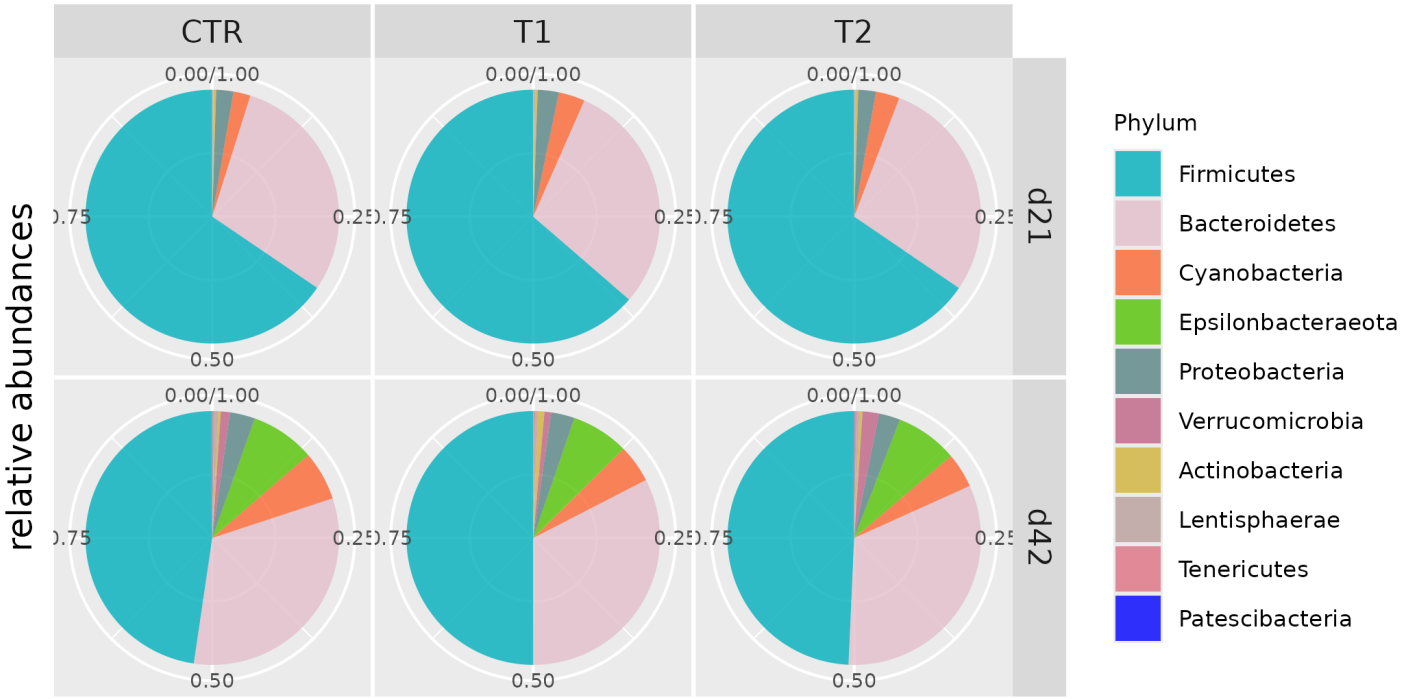


- Significant effect of the **time** → greater alpha diversity at d42 compared to d21
- Effect of the **treatment** → significant for Simpson e Shannon in T1 compared to CTR. No differences between T2 e CTR



- No significant effect of the **time**
- No significant effect of the **treatment**

Cecal microbiota – relative abundance (phylum)



- Relative abundance similar among treatments within timepoint
- Firmicutes the most abundant phylum, followed by Bacteroidetes
- Phylum composition aligned with composition reported in literature

Cecal microbiota – different OTUs

Day 21

T1 vs CTR

- Lactobacillus
- Bacteroides
- Eggerthella
- Holdemania
- Angelakisella
- Staphylococcus

T2 vs CTR

- Lactobacillus
- Bacteroides
- Angelakisella

Day 42

T1 vs CTR

- uncultured bacterium
- Ruminococcaceae UCG-014
- Lactobacillus
- Bacteroides
- Parasutterella
- Klebsiella
- UBA1819

T2 vs CTR

- uncultured bacterium
- Ruminococcaceae UCG-014
- Lactobacillus
- Anaerostipes
- Negativibacillus
- Klebsiella

- Ruminococcaceae UCG-009
- Balutia
- Defluviitaleaceae UCG-011
- [Ruminococcus] torques group

- Blautia
- Olsenella
- Jeotgalibaca
- [Eubacterium] xylanophilum group
- Ruminococcaceae UCG-005
- Roseburia
- Lachnospiraceae UCG-008
- Ruminococcaceae V9D2013 group
- [Ruminococcus] torques group

Cecal microbiota – different OTUs

Day 21

T1 vs CTR

- **Lactobacillus**
- **Bacteroides**
- Eggerthella
- Holdemania
- Angelakisella
- Staphylococcus

T2 vs CTR

- **Lactobacillus**
- **Bacteroides**
- Angelakisella

Day 42

T1 vs CTR

- uncultured bacterium
- Ruminococcaceae UCG-014
- **Lactobacillus**
- **Bacteroides**
- Parasutterella
- Klebsiella
- UBA1819

T2 vs CTR

- uncultured bacterium
- Ruminococcaceae UCG-014
- **Lactobacillus**
- Anaerostipes
- Negativibacillus
- Klebsiella

- Opposite result compared to literature for **Lactobacillus** →
  - Chickens (Wu et al., 2018; http://dx.doi.org/10.3382/ps/pey268)
  - Piglets (Waititu et al., 2017, https://doi.org/10.1017/S1751731117001276)
  - Rats (Ding et al., 2022; https://doi.org/10.3389/fnut.2022.820799)

- Rumi… UCG-…
- Balut…
- …torques group

- [Eubacterium] xylanophilum group
- …nococcaceae UCG-005
- …buria
- …nospiraceae UCG-008
- Ruminococcaceae V9D2013 group
- [Ruminococcus] torques group

- Main observed effects with T2 (highest dosage)
  - Improved performance (BW, ADG) at the initial stage of life (d21)
  - Beneficial effect on jejunal morphology
  - Increased TJ gene expression

- **Yeast nucleotides administration can potentially benefit gut health of younger animals**
- **Mode of action to be unravelled**
- **Further evaluations under challenging conditions**

Thanks for your attention!

Contacts

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