

75th Annual Meeting of the
European Federation of Animal Science

Session 95

Evaluation of dietary inclusion of *Tenebrio molitor* larvae on gut microbiota modulation in growing pigs

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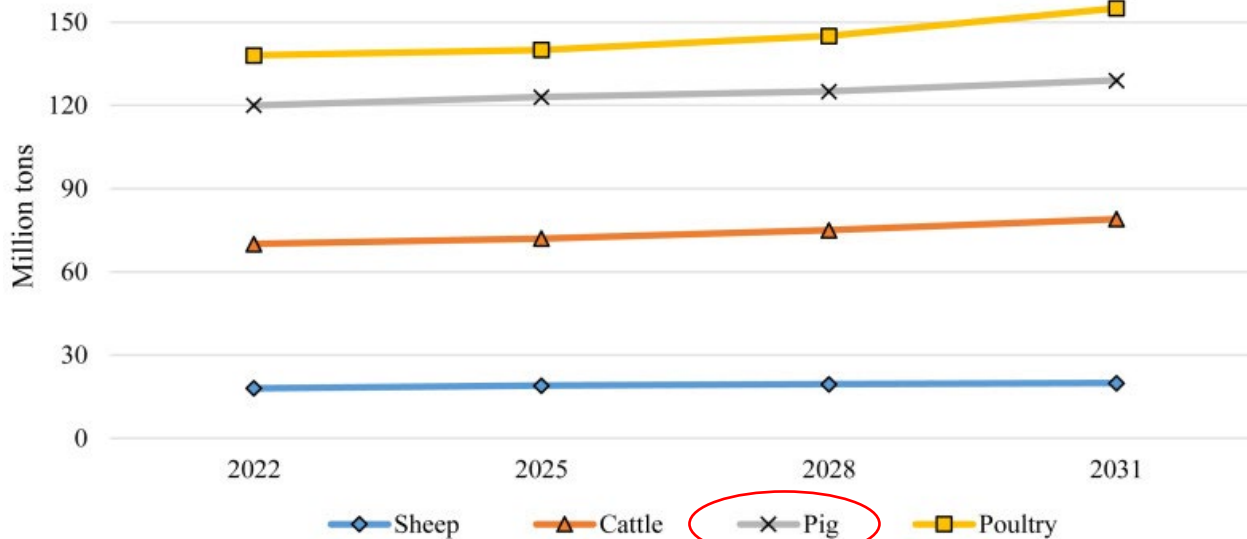
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Introduction



Projected meat production volume by animal species



OECD-FAO; Mateos et al., 2024



European Green Deal: food systems fair, healthy environmentally-friendly

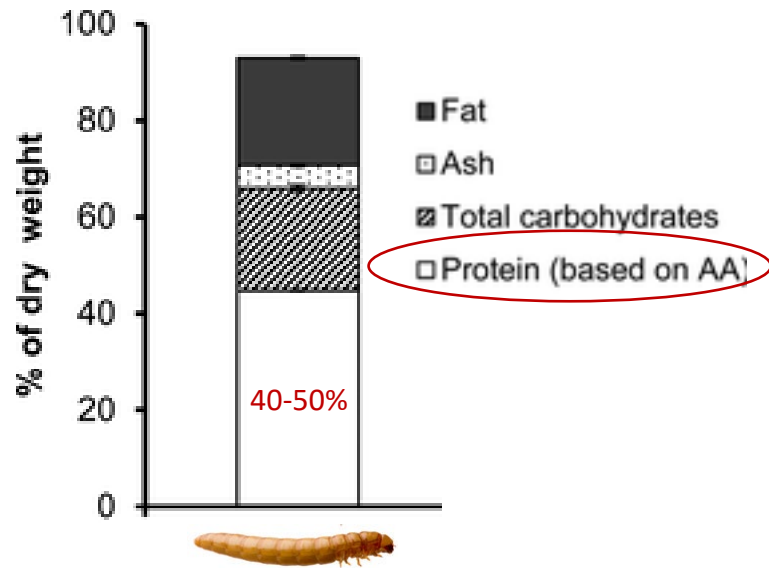
Insects:
Alternative protein source for animal nutrition (Reg. (UE) 2021/1372).



Tenebrio molitor larvae

- EFSA opinion (Jan 2021)

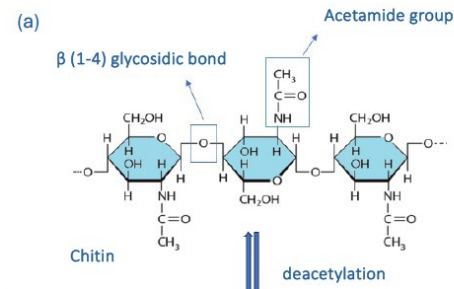
NUTRITIONAL PROFILE



(Janssen et al., 2017)

BIOACTIVE MOLECULES

➤ Chitin



➤ Lauric acid

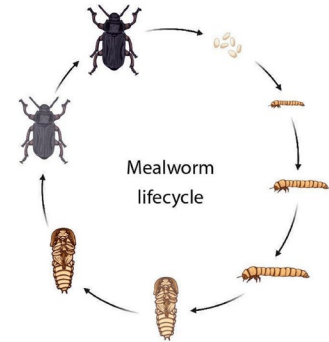


➤ Antimicrobial peptides (AMP)



(Shin et al. 2019; Baek et al. 2019; Gasco et al., 2020; Jantzen da Silva Lucas et al. 2020; Ferri et al., 2024)

EASY REARING & SHORT LIFE CYCLE



(Pinotti et al., 2019; Dreyer et al., 2021; Oppert et al., 2023)

Tenebrio molitor larvae as an alternative protein source in swine nutrition

The supplementation of dried mealworms in pig diets has shown promising results in:
(Jin et al., 2016; Ko et al., 2020; Zacharis et al., 2023)



GROWTH PERFORMANCE



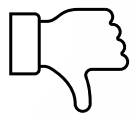
NUTRIENT DIGESTIBILITY



PROTEIN UTILIZATION



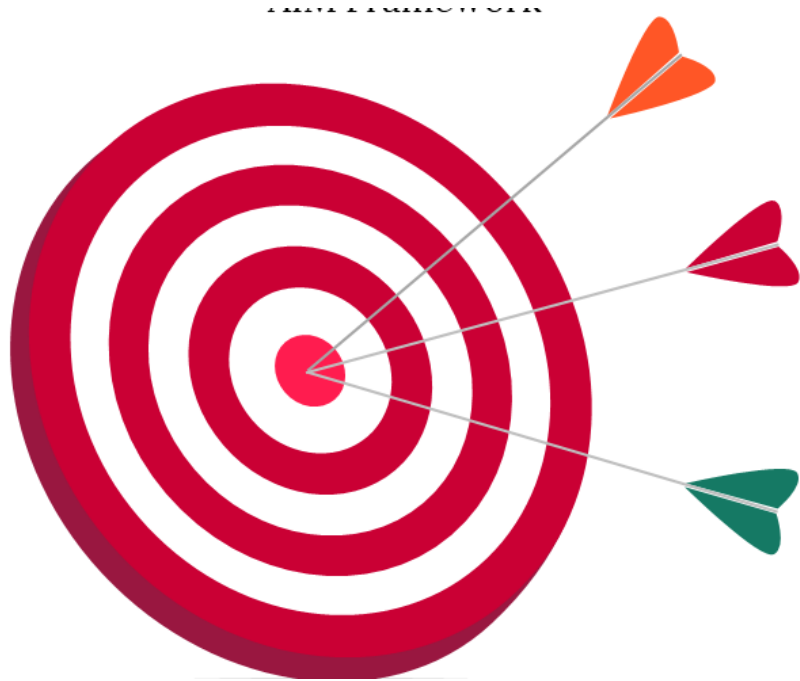
WHAT IS THE OPTIMAL INCLUSION % OF INSECT MEALS IN PIG DIET?



10 % *T. molitor* dietary supplementation reported negative effect on the growth performance
(Meyer et al., 2020)

Aim

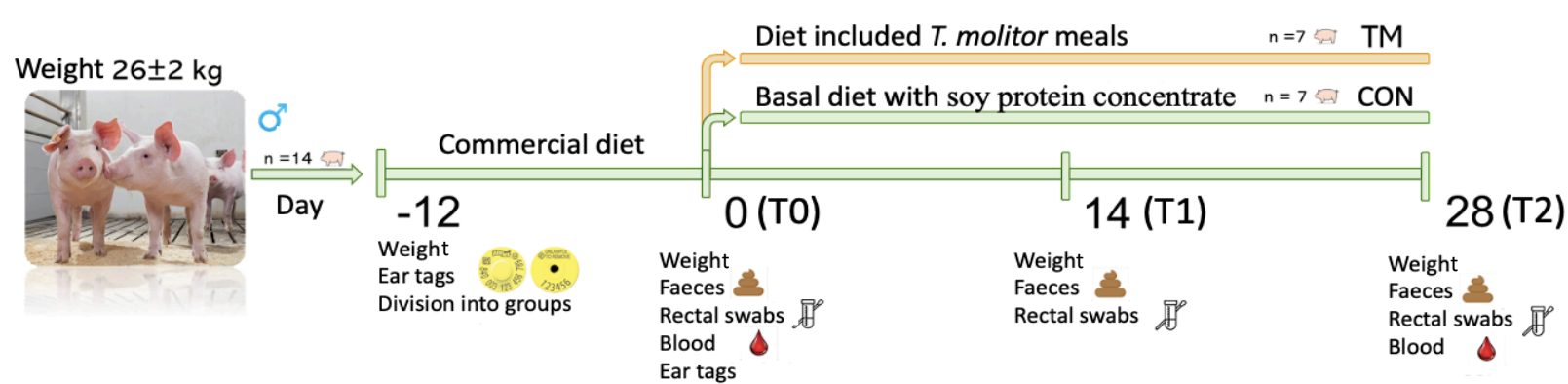
The aim of this preliminary study was to evaluate the replacement of fermented soy protein concentrate with 5 % of *Tenebrio molitor* meals on performance, diet digestibility, and gut microbiota of growing pigs.



- Growth performance (BW, ADG, ADFI, FCR) and health status
- Apparent nutrient digestibility (for proteins and lipids)
- Blood metabolites
- Modulation of gut microbiota

Experimental design

Materials and methods



Principal nutrients	Experimental diets	
	CON	TM
Dry matter, %	91.39	91.60
Crude protein, %	16.60	16.07
Crude fiber, %	4.94	4.58
Crude fat, %	6.72	6.55
Ash, %	5.78	5.79

Ingredients, as % of Fed Basis	CON	TM
Fermented soy protein concentrate	4.000	-
Soybean oil	1.000	-
<i>Tenebrio molitor</i> meal		5.000

The experimental trial was approved by the Animal Welfare Body of the University of Milan (Authorization No. 4/2023) and performed in compliance with European legislation (Dir. EU 2010/63).

Zootechnical evaluation and diet digestibility

Materials and methods

✓ Growth performance:

Body weight (**BW**), Average Daily Gain (**ADG**), Average Daily Feed Intake (**ADFI**)

✓ Protein and lipid digestibility: Acid insoluble ash method

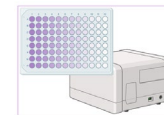
Nitrogen content (Kjeldahl method)

Lipid concentration- EE (AOAC 930.15; AOAC 2001.11; AOAC 2003.05 method)

$$\text{Apparent Nutrient Digestibility (\%)} = 100 \times \left(1 - \frac{\%AIA \text{ in feed}}{\%AIA \text{ in faeces}} \times \frac{\%Nutrient \text{ in faeces}}{\%Nutrient \text{ in feed}} \right)$$

✓ Blood samples:

Il-6 → commercial enzymatic immunoassay kit (Porcine Interleukin 6, IL-6 ELISA Kit®)

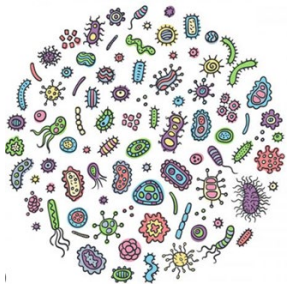


Total proteins, Albumin, Globulin, A/G, Urea → multiparametric autoanalyzer for clinical chemistry (ILab 650)



Microbiota analysis

Materials and methods

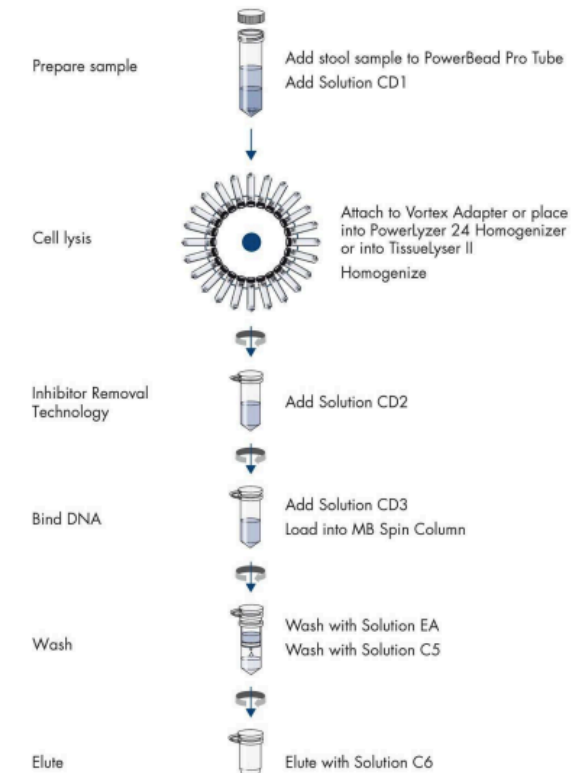


✓ Amplification and sequencing region V3-V4
rRNA 16S gene (Cremonesi et al., 2022)



✓ Diversity index: alpha and beta diversity
Chao1 and ACE (richness), Shannon, Simpson and Fisher alpha
(diversity), Simpson E and Pielou (evenness)

QIAamp PowerFecal Pro DNA Kit Procedure



Bioinformatic and biostatistic analysis: Micca and R software (Biscarini et al., 2012)



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E SCIENZE ANIMALI

Zootechnical performance

Results

Item	CON	TM	p values		
			Treatment	Time	Treatment×Time
BW, kg			0.7666	<0.0001	0.5885
d 0	26.57 ± 2.92	26.01 ± 2.67			
d 14	36.20 ± 3.03	35.17 ± 3.92			
d 28	48.44 ± 3.41	48.41 ± 4.52			
ADG (kg)			0.6478	0.0003	0.2846
d 0-14	0.688 ± 0.09	0.654 ± 0.14			
d 14-28	0.874 ± 0.12	0.946 ± 0.11			
d 0-28	0.781 ± 0.06	0.800 ± 0.09			
ADFI (kg)			-	-	-
d 0-14	1.54	1.60			
d 14-28	2.30	2.24			
d 0-28	1.92	1.92			

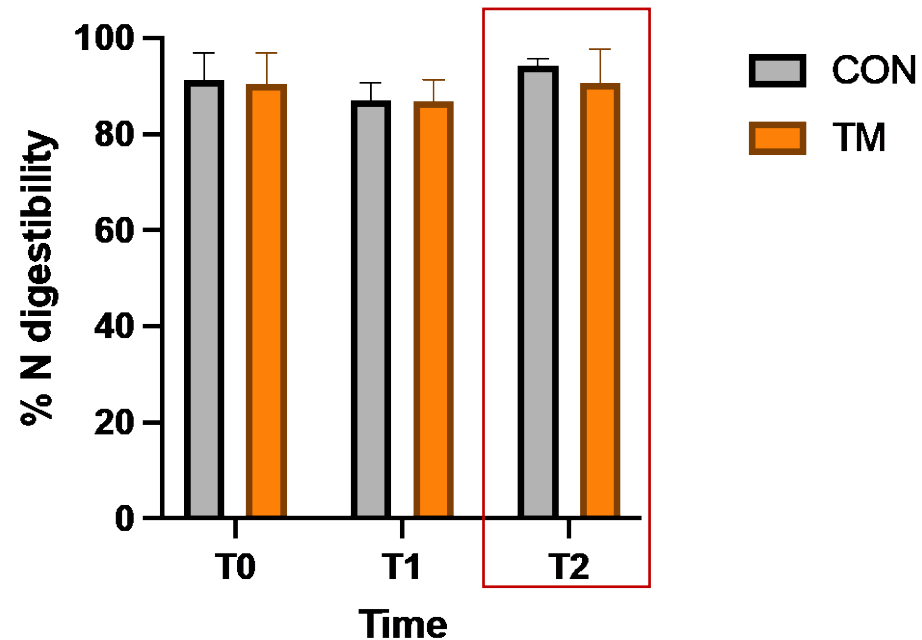
T. molitor inclusion (TM, n = 7) or with soy protein (CON, n = 7). BW: body weight; ADG: average daily gain; ADFI: average daily food intake. Data are shown as mean ± standard deviation (SD).



Protein and lipid digestibility

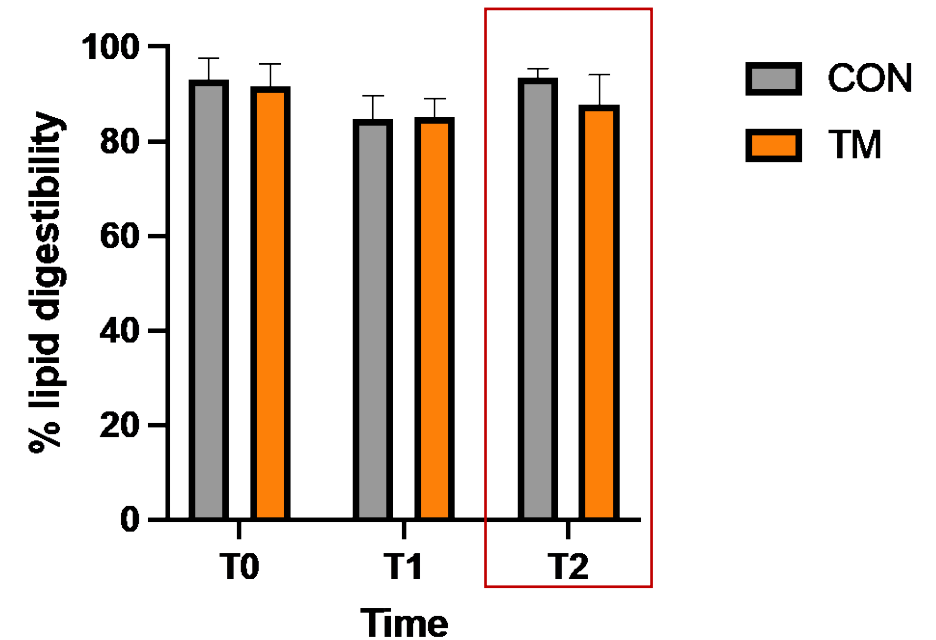
Results

Apparent total digestibility of nitrogen



$p > 0.05$

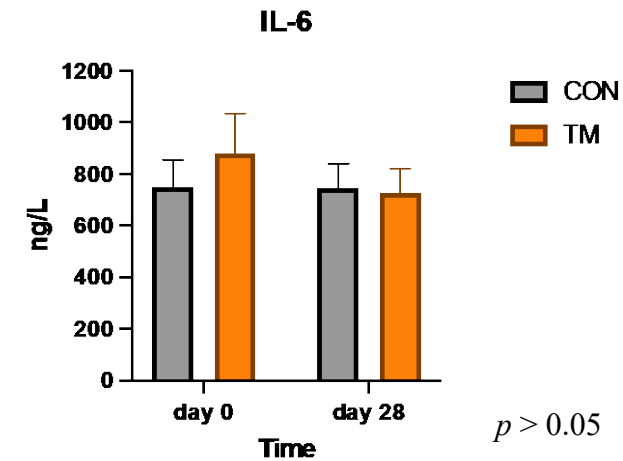
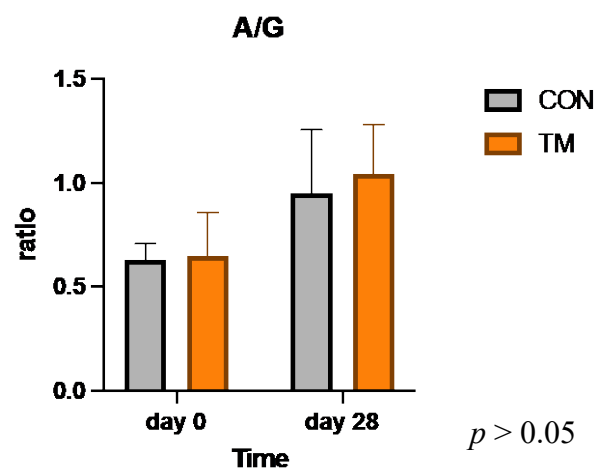
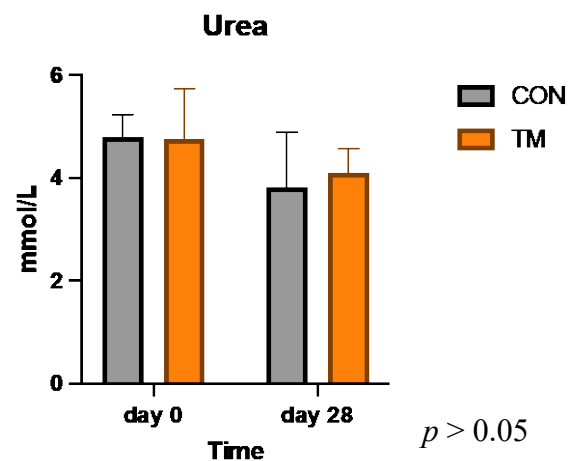
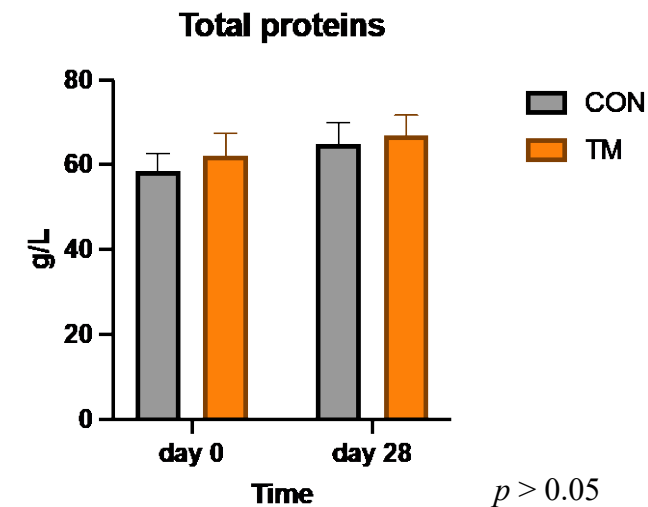
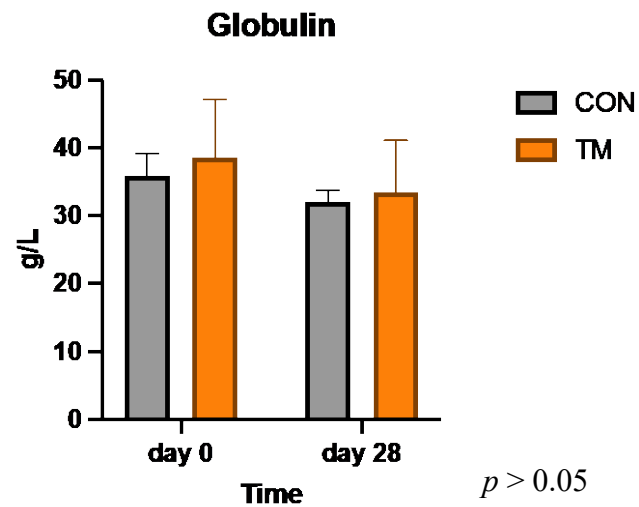
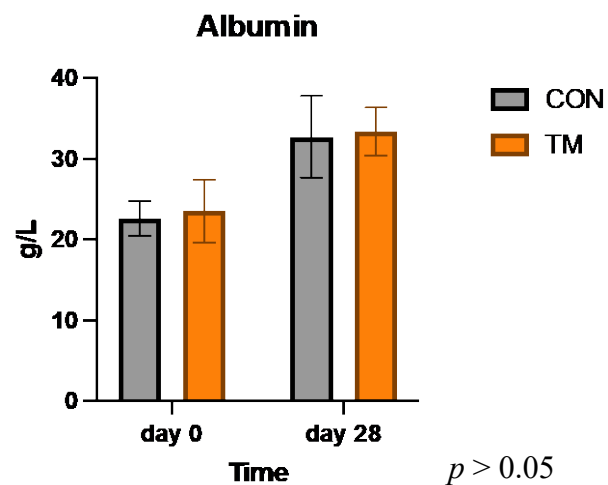
Apparent total digestibility of lipids



$p > 0.05$

Blood serum metabolites

Results



Microbiota composition and community diversity

Results

Ratio:

Firmicutes/Bacteroidetes: not variation

✓ Firmicutes / Euryarchaeota

Euryarchaeota sequences were associated with the hydrogenotrophic methanogens (Feehan et al. 2023).

Short chain fatty acids producers

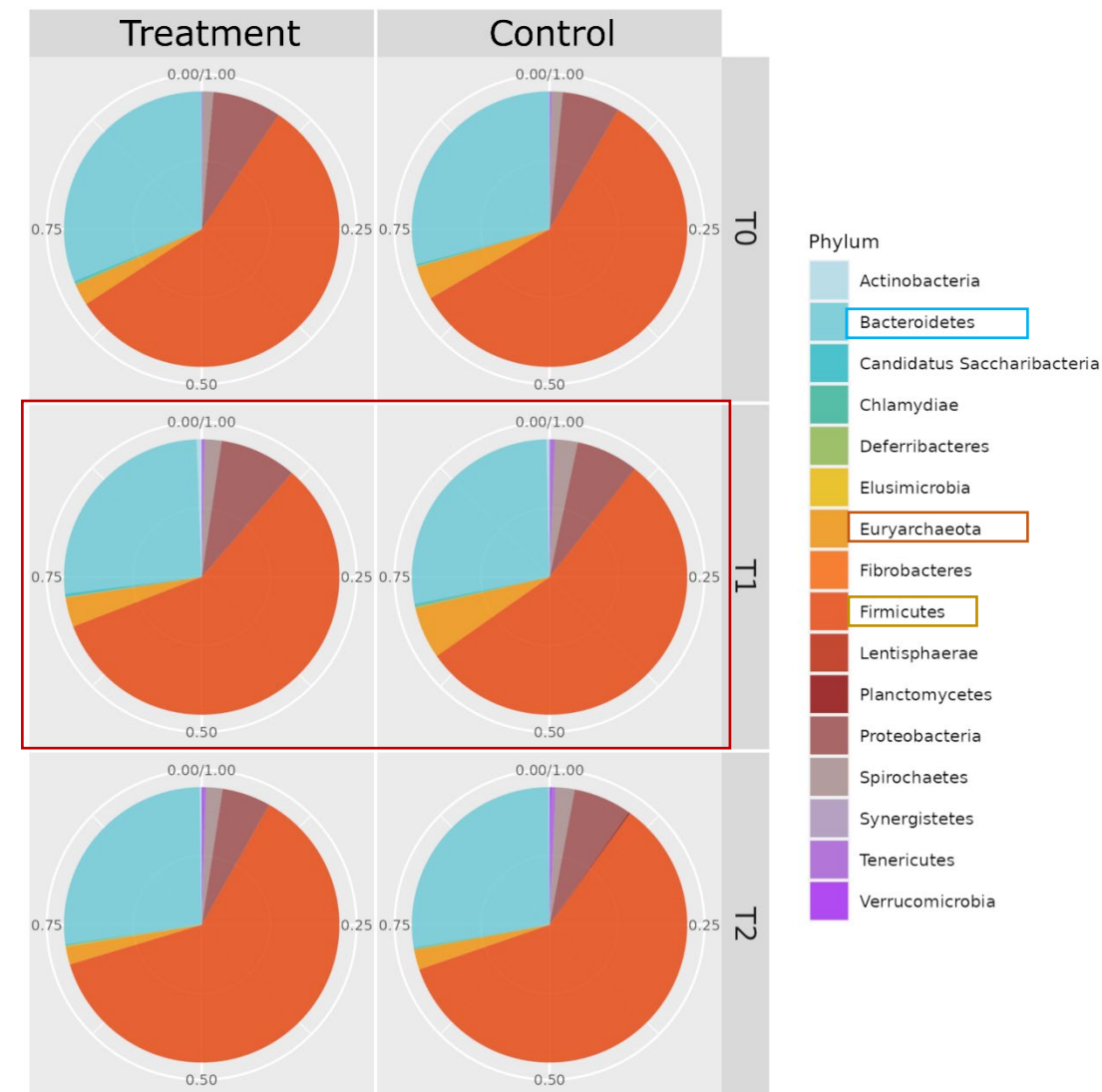
At the genera level:

DAY 14

Acinetobacter, *Actinobacillus*, *Alloprevotella*, *Blautia*, *Butyrivibrio*, *Clostridium* IV, *Clostridium* XIVa, *Eubacterium*, *Methanosphaera*, *Phascolarctobacterium*, *Prevotella*, *Roseburia*, *Ruminococcus* and *Turicibacter* spp. TM > CON (p value < 0.05).

DAY 28

Asteroplasma TM < CON (p value < 0.05)

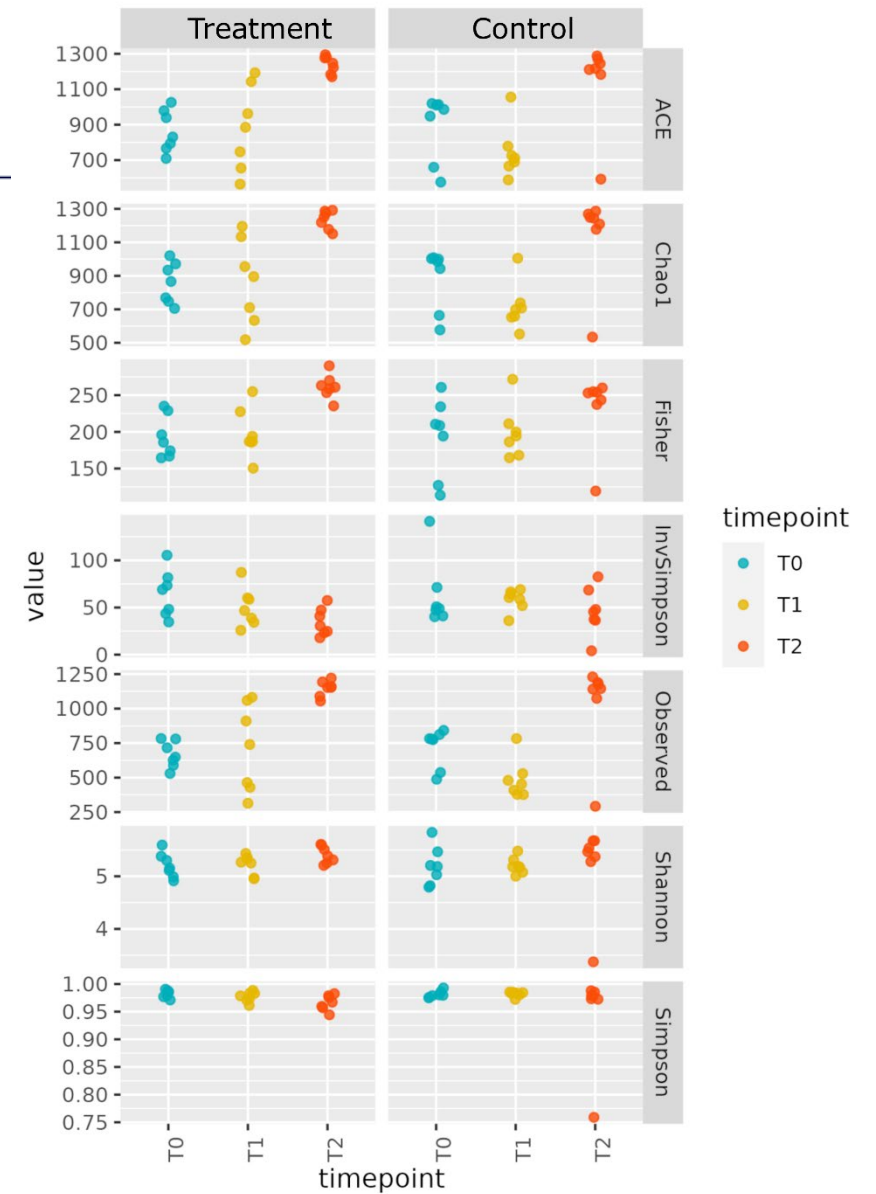


Diversity index

Results

Alpha diversity:

- Indices: ACE, Chao1, Fisher, InvSimpson, observed, Shannon, Simpson
- Results were not significantly different at days 14 and 28.

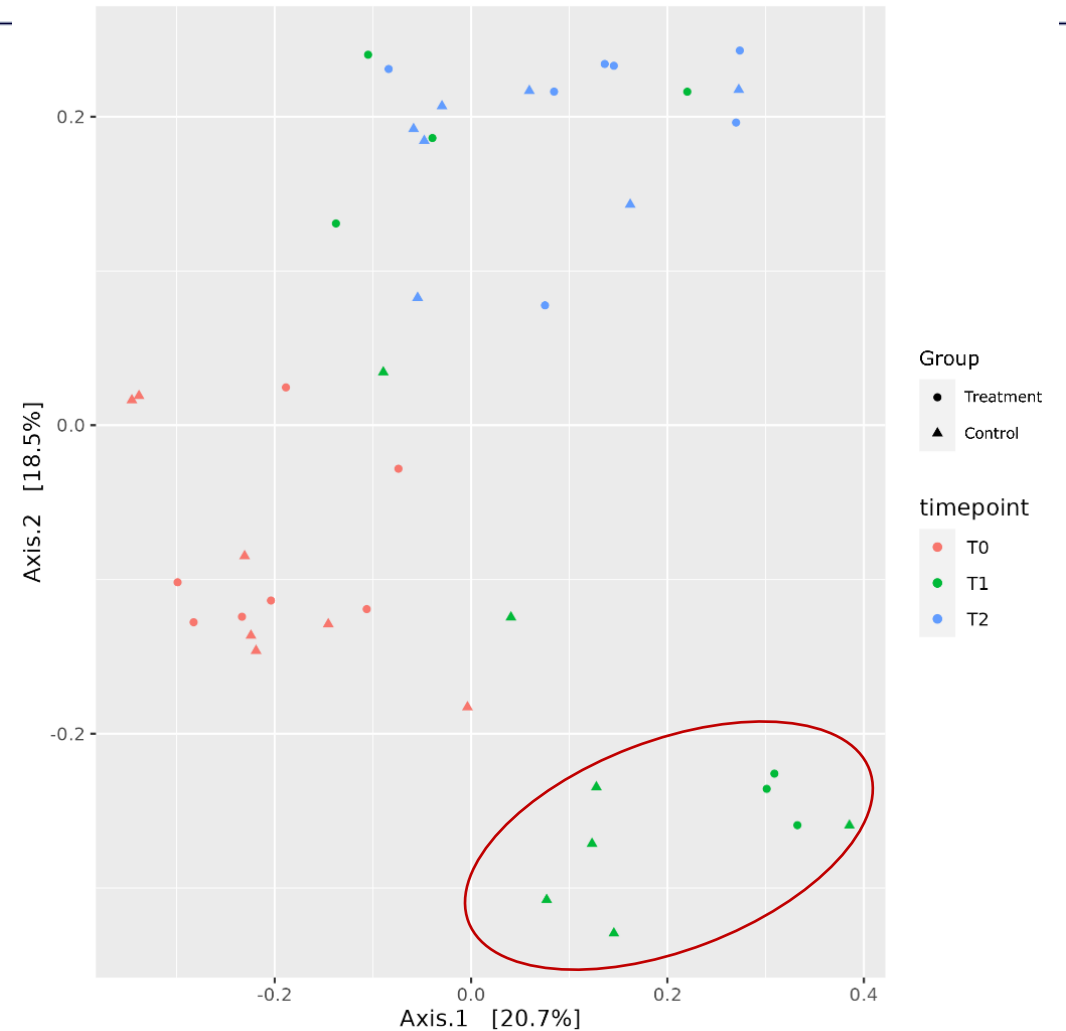


Diversity index

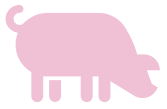
Results

Beta diversity:

- Bray–Curtis index
- Results showed a significant difference considering the entire period of 28 days (PERMANOVA, R^2 0.0384, p value = 0.0260)



Conclusion



Our results showed that is possible to replace fermented soy protein concentrate with 5% *Tenebrio molitor* meal without affecting growing pigs' growth and intestinal health.



✓ Insect meals have been shown to modulate specific bacteria that may contribute to the sustainability of livestock farms as well as to animal health



Administering *T. molitor* meal in an earlier stage, such as during **weaning phase**, could lead to a stable modulation of the microbiota



Given the encouraging results, we have planned study on weaned piglets with a 42-day observation period in accordance with EFSA guidelines.





Thank you for the attention

