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Effects of two blends of phytoextracts on growth and gut health of weaning pigs to replace zinc oxide

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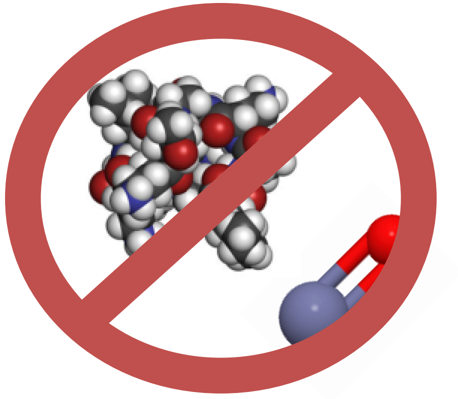
Introduction

Early weaning

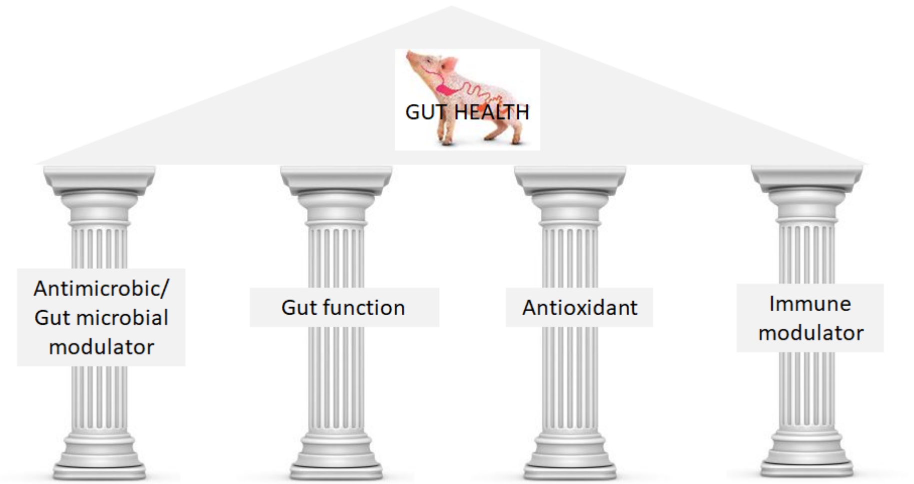
- Social stress
- Change of diet
- Change of the environment
- Drop of acquired immunity level

- Low capacity to absorb fluid
- Age-related expression of specific receptors for bacteria adhesion
- Lack of a complete immune competence

↓ Feed intake
↓ Intestinal eubiosis
↑ Diarrhea
↑ Mortality
↓ Performance



Feed additive and gut health



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Introduction

Natural and natural identical essential oils compounds has shown promising results

The most used bioactive substances are cinnamaldehyde, thymol, carvacrol, eugenol and capsaicin.

Reduction of the inflammatory process

Antibacterial properties

Improve the gut morphology parameters

Improve digestibility

→ Maintaining the gut health and the growth of post-weaning pigs

- Cinnamaldehyde → has bactericidal effect against *E. coli* interfering with the DNA and RNA synthesis (Pereira et al., 2021)
- Thymol and carvacrol → have known bactericidal activity *E. coli* linked to the lysis of the bacterial cytoplasmic membrane (Xu et al., 2008)
- Eugenol → has a synergistic bacteriostatic effect against *E. coli* when combined with cinnamaldehyde, thymol and carvacrol (Pei et al., 2009)
- A mixture of carvacrol, thymol and cinnamaldehyde → was able to maintaining the antioxidant balance and reducing gut inflammation (Rebucci et al. 2022)

However, there is inconsistency in the literature regarding the effectiveness of blend of natural and natural identical essential oils compounds, possibly due to different formulations and lack of specific data (Huang & Lee 2018).

Pereira et al., (2021). Biomolecules, 11: 302. Xu et al., (2008) Letters in Applied Microbiology 47: 174–179. Pei et al., (2009). Journal of Food Science 74: M379-M383. Rebucci et al., (2022). Livestock Science 263: 104959. Huang, C. M., & Lee, T. T. (2018). *Asian-Australasian journal of animal sciences*, *31*(5), 617.

Introduction

Organic acids have been widely investigated for their potential role in controlling the gut environment and gut health in post-weaning pigs

Cx	Trivial name	Chemical formula
C1	Formic	$HCOOH$
C2	Acetic	CH_3COOH
C3	Propionic	CH_3CH_2COOH
C4	Butyric	$CH_3CH_2CH_2COOH$
C5	Valeric	$CH_3CH_2CH_2CH_2COOH$
C6	Caproic	$CH_3CH_2CH_2CH_2CH_2COOH$
C7	Enanthic	$CH_3CH_2CH_2CH_2CH_2CH_2COOH$
C8	Caprylic	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2COOH$
C9	Pelargonic	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$
C10	Capric	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$
C11	Undecylic	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$
C12	Lauric	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$

Short

Medium

Following the approach called «multi-hurdled» proposed in humans (Rostami et al., 2016), a mixture of short and medium fatty acids (FAs) or a mixture of natural and naturally identical essential oils with FAs could represent an additional strategy

Item	CTR1	AGP	OA1	OA2	P value	REF
ADG, g/d	295ab	281b	314ab	332a	**0.03**	Long et al., 2018
FE	502	478	476	488	0.48	
Diarrhoea rate	3.58a	1.59b	0.60b	0.40b	**<0.01**	
Blood, IgM, g/l	0.57b	0.65ab	0.97a	0.70ab	**0.05**	
Escherichia coli, feaces	4.50a	2.60b	3.36b	2.87b	**0.04**	
Total volatile fatty acid	2.88b	4.40a	5.00a	4.96a	**<0.01**	
Acetic acid	1.33b	1.76a	2.10a	2.03a	**<0.01**	
Propionic acid	0.70b	1.26a	1.21a	1.32a	**<0.01**	

	NC	PC	AGPs	OAMF1	OAMF2	P value	REF
ADG	163 a	164 a	181 b	182 b	177 ab	**0.034**	Han et al., 2020
FE	45	356	378	375	360	0.594	
Faecal score	1.96 a	1.93 a	1.46 d	1.60 c	1.71 b	**< 0.001**	
IgM (g/L)	2.25 a	2.35 ab	2.37 bc	2.42 c	2.32 ab	**0.009**	
IgG (g/L)	18.91 a	18.56 a	19.44 a	21.04 b	21.44 b	**< 0.001**	
Megasphaera	0.30a	0.57a	1.06b	0.46a	0.54a	**0.038**	
Faecalibacterium	0.22	0.22	0.56	0.16	0.21	**0.055**	

- AGP=CTR + 10 mg/kg zinc bacitracin, 5 mg/kg colistin sulphate and 5 mg/kg olaquindox;
- OA1= synergistic blend of free and buffered short chain fatty acids (mainly formic acid, acetic acid and propionic acid) combined with MCFA:
- OA2= synergistic blend of a phenolic compound, slow release C12, target release butyrate and sorbic acid, MCFA and OA
- PC=positive control, basal diet, challenge with *E. coli* K88;
- AGP= antibiotic growth promoter, challenged;
- OAMF1= a synergistic fat-coated blend of a phenolic compound, slow release C12 (ester of lauric acid), targeted release butyrate, MCFAs (caproic, caprylic, capric, and lauric acid) and sorbic acid;
- OAMF2=blend of buffered short chain fatty acids (formic acid, ammonium formate, acetic acid, propionic acid, butyric acid, lactic acid, citric acid, and sorbic acid) combined with MCFAs.

The combination of cinnamaldehyde, thymol, carvacrol, and organic acids may have synergistic effects in controlling post weaning diarrhoea and improving intestinal health in post-weaning piglets.

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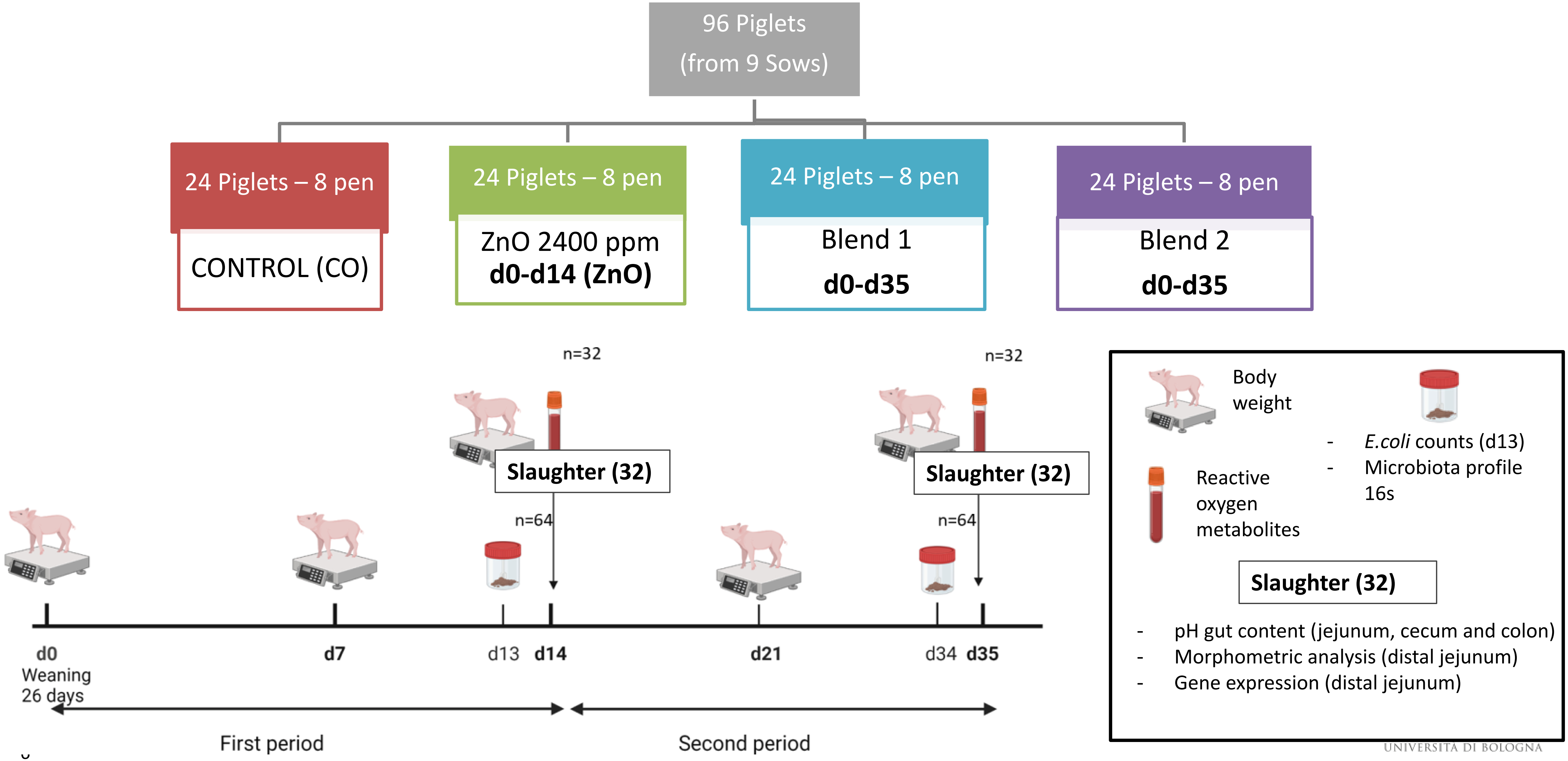
Hypothesis

The hypothesis underlying this study is that the use of mixtures of natural and natural identical essential oils alone and mixed with organic acids, could be a good strategy to replace ZnO at pharmacological doses in post-weaning piglets thanks to their different modes of action.

Aim

This study aimed to evaluate the effect of the administration and the modes of action of two blends of natural and natural identical essential oils containing mainly cinnamaldehyde, thymol, carvacrol and eugenol alone or in combination with a mixture of organic acids compared to the pharmacological dose of ZnO on the health, growth performance, and gut health, including faecal microbial profile of weaned pigs

Material and Method: Experimental design



The blends composition

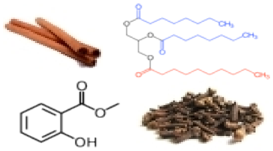
Blend 1: 150 g/100 kg feed

- cinnamaldehyde (natural identical),
- cassia oil (titrated in cinnamaldehyde),
- ajowan essential oil (titrated in thymol),
- essential oil of clove (titrated in eugenol)

Total extract of 85.2 g/kg including:
40g/kg of cinnamaldehyde
10.65 g/kg of thymol,
9.84 g/kg of carvacrol,
8.31 g/kg of p-Cymene,
8.06 g/kg of eugenol,
4.57 g/kg of y-Terpinene,
1.91 g/kg diallyl
196 disulphide
1.88 g/kg beta-caryophyllene.

The product was microencapsulated using a patented system that uses natural polysaccharides.

Blend 2: 200g/100 kg feed



- medium chain fatty acids in ester form,
- eugenol (natural identical),
- cinnamaldehyde (natural identical)
- methyl salicylate (natural identical).

The total extract was 100g/kg including:
88g/kg of cinnamaldehyde
9 g/kg of eugenol
3 g/kg of methyl salicylate.

The mono and diglycerides of fatty acids were 400 g/kg including butyric, propionic, capric and caprylic acid esters, free glycerol, free fatty acids and water.

The product was microencapsulated using 340 g of refined and hydrogenated palm oil and 160 g of silicic acid.

Results: Faecal score

Faecal score	Diet				SEM	p-Value
	CO	ZnO	Blend1	Blend2		
d0-d7	2.3	2.21	2.36	2.21	0.07	0.32
d7-d14	2.1	2.04	2.12	2.07	0.04	0.45
d0-d14	2.2	2.13	2.25	2.14	0.04	0.13
d14-d28	2.0	2.02	2.02	2.01	0.00	0.95
d0-d35	2.13	2.08	2.14	2.10	0.02	0.40

Animals were in a good state of health, as evidenced by the faecal score data which were under the threshold of diarrhoea throughout the whole trial

Results: growth performance

Item	Diet				SEM	p-Value
	CO	ZnO	Blend1	Blend2		
Body weight, g						
d0	7077	7066	7077	7041	325	1.00
d7	7811	7762	7970	7660	341	0.93
d14	10336	10124	10003	9346	403	0.31
d28	17720	17433	17243	16144	587	0.13
d35	22495	22235	22235	20541	722	0.12
Average daily gain, g/d						
d0-d7	104.2	99	125.4	91.4	17.7	0.45
d7-d14	361 **A**	337 **A**	290 **AB**	240 **B**	26.7	**<0.0001**
d0-d14	233 **A**	218 **A**	208 **AB**	165 **B**	14.1	**0.002**
d14-d28	520	517	531	484	22.8	0.38
d28-d35	682	682	716	630	32.5	0.31
d14-d35	574	574	592	532	22.7	0.2
d0-d35	438	432	431	387	17.7	0.1
Feed intake, g/d						
d0-d7	137	142	166	128	14.5	0.32
d7-d14	467 **A**	436	448	364 **B**	25.8	**0.05**
d0-d14	302 **a**	289 **ab**	307 **a**	246 **b**	16.5	**0.05**
d14-d28	813	787	811	714	34.2	0.17
d28-d35	1135	1172	1184	1065	50	0.35
d14-d35	921	915	935	831	35.7	0.18
d0-d35	673	665	684 **a**	597 **b**	25.5	**0.09**
Feed to gain						
d0-d7	1.37	1.59	1.33	1.47	0.1	0.27
d7-d14	1.3 **A**	1.29 **A**	1.56 **AB**	1.66 **B**	0.08	**0.01**
d0-d14	1.3 **A**	1.32 **A,b**	1.47 **b,C**	1.53 **C**	0.05	**0.01**
d14-d28	1.57	1.53	1.52	1.49	0.04	0.43
d28-d35	1.67	1.72	1.64	1.7	0.03	0.38
d14-d35	1.61	1.6	1.57	1.57	0.03	0.55
d0-d35	1.54	1.54	1.57	1.55	0.02	0.82

The CO group and the ZnO did not differ in terms of ADG, FI and G:F, and highlighted that ZnO has no effect on the performance when piglets are under healthy conditions (Hung et al., 2020; Liu et al., 2020)

The Blend 1 did not significantly improve the ADG or affect the FI compared with the CO and ZnO diets. However, considering the F:G from d0 to d14 the Blend1 tended to have a higher value compared with the CO group.

The Blend2 reduced the growth performance compared to both the CO and the ZnO groups from d0-d14.

- not optimal encapsulation
- too high dose

Hung et al., 2020; Antibiotics 9: 463. Liu et al., 2020; Animals 10: 757

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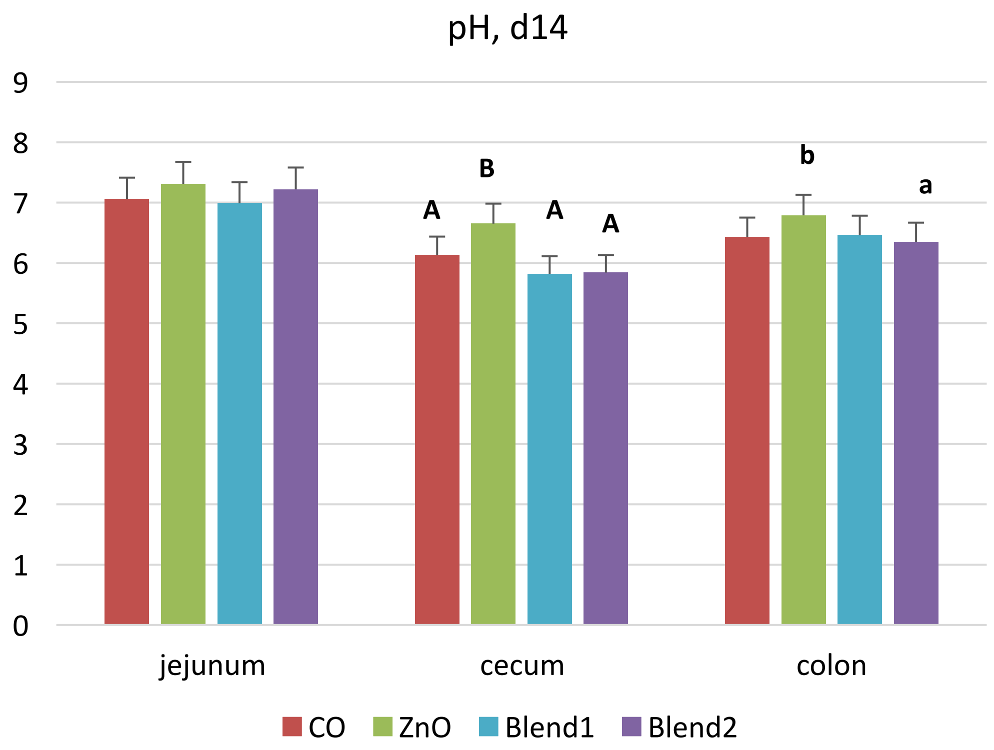
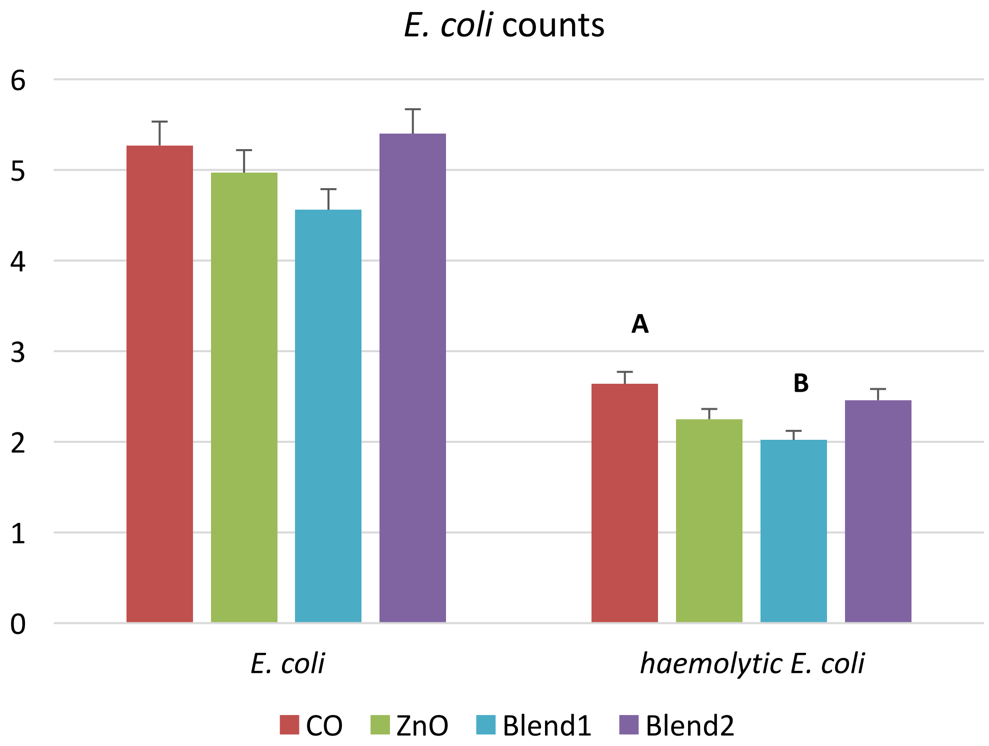
Results: Intestinal pH and E.coli counts





The use of ZnO increased the pH in the large intestine at d14 while its effect was not observed at d35 when the ZnO was not included in the diet.

The increase in the pH in can be associated with a reduction in the production of short-chain fatty acids by intestinal bacteria (Heo et al., 2013).

The Blend1 reduced the haemolytic count in the faeces of piglets at d13 compared with the CO (*P*=0.01).

Thymol, carvacrol, eugenol and cinnamaldehyde can inhibit the proliferation of the pathogens by interfering with cytoplasmic membranes and energy metabolism (Omonijo et al., 2018).

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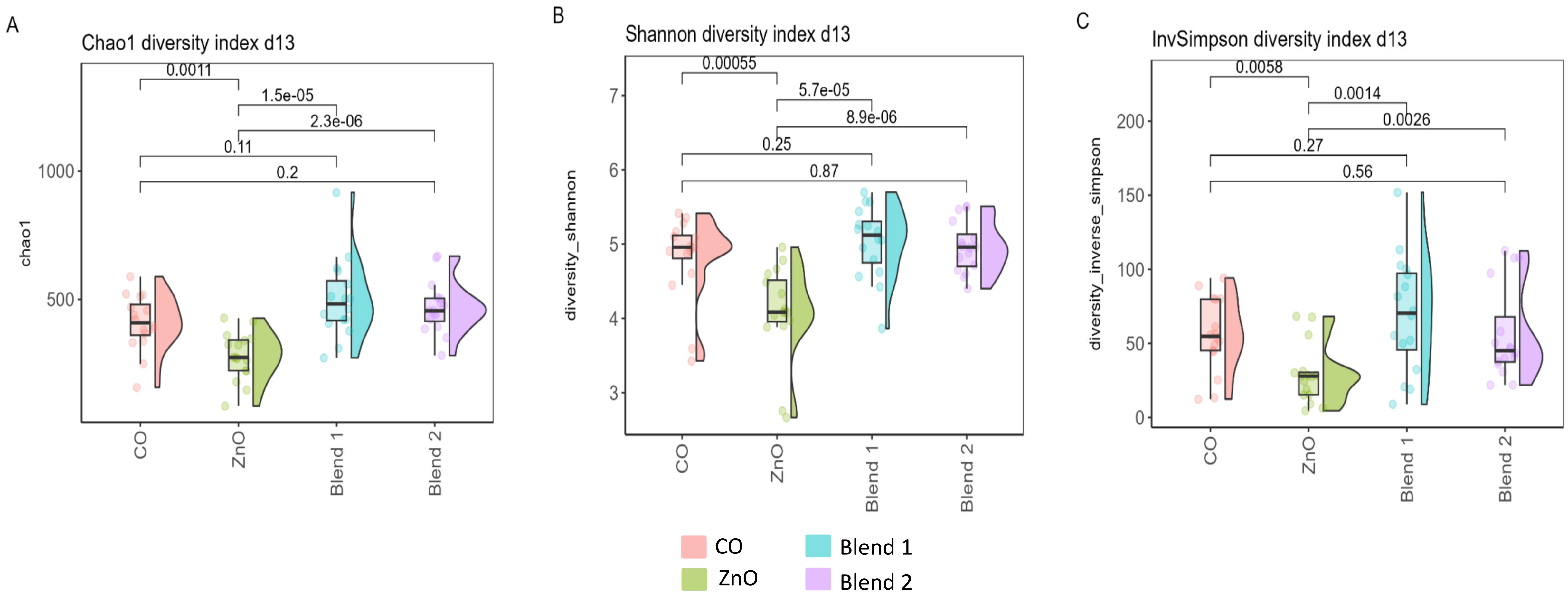
Heo et al., 2013, Journal of animal physiology and animal nutrition 97: 207-237. Omonijo, F.A. 2018.. Animal Nutrition 4: 126-136.

Results: Microbial Alpha diversity



At d13, the ZnO group had a significantly lower alpha diversity, for all the indices considered while no differences were observed at d34

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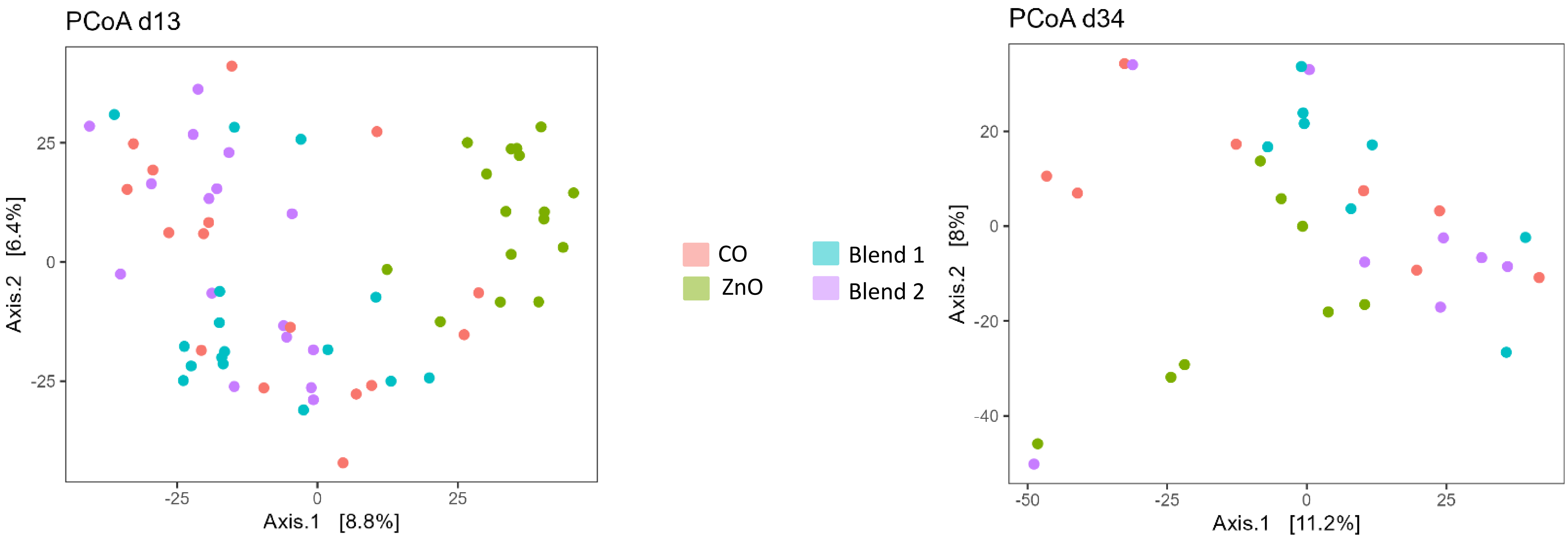
Results: Microbial Beta diversity



At d13, ZnO group had a significantly different bacterial structure compared with the CO (R^2=0.09, *P* adj. <0.001), Blend1 (R^2=0.11, *P* adj. <0.001) and Blend2 (R^2=0.11, *P* adj. <0.001) groups.

At d34, the bacterial structure continued to be affected by the diet (R^2=0.18, *P* = 0.046); however, the differences among the groups were mostly driven by the comparison between ZnO and Blend1 (R^2=0.1, *P* adj.=0.01) and no differences were observed for the other comparisons.

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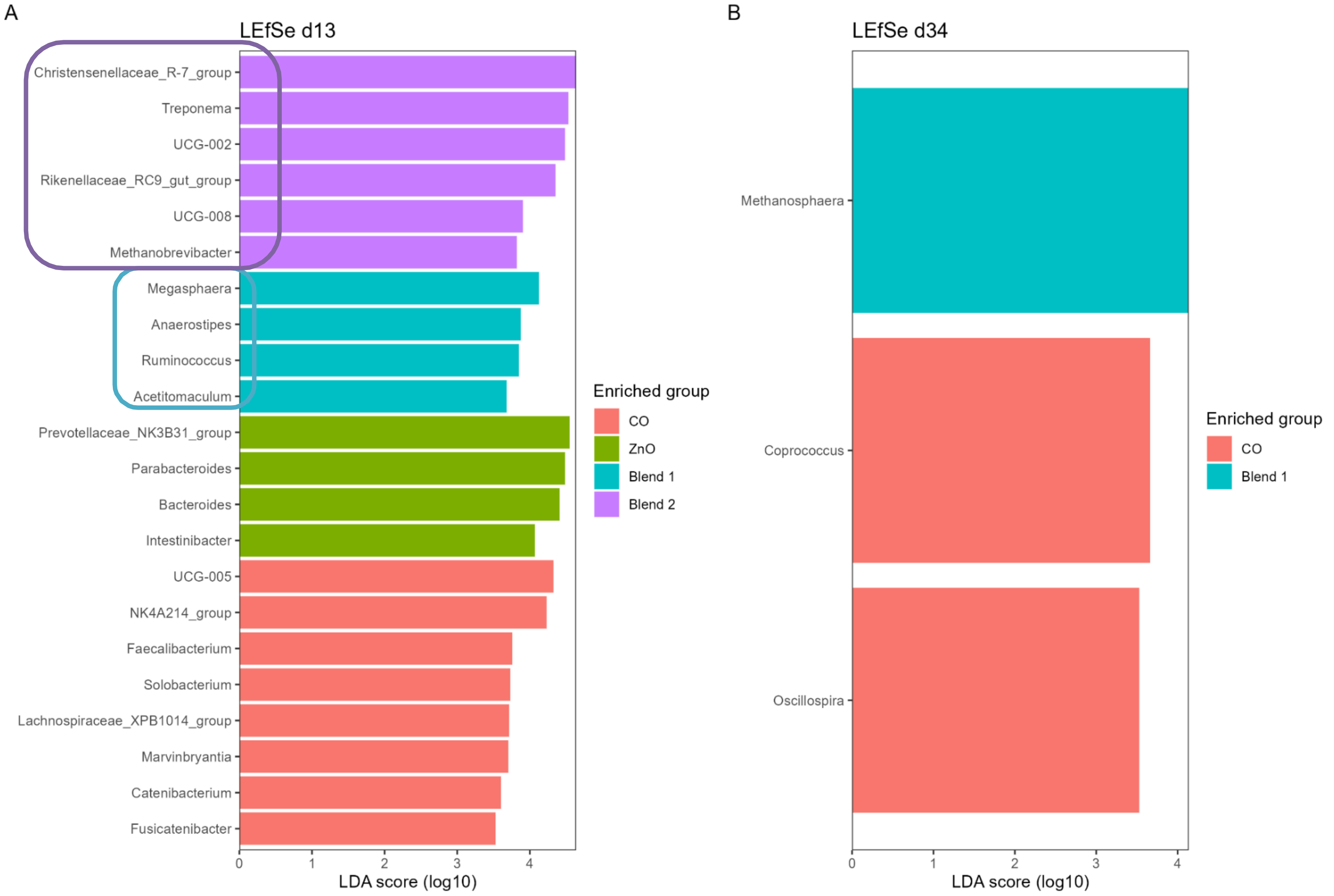
Results: Bacterial marker



Blend1 promoted the abundance of *Acetitomaculum*, which is an acetate-producing genus (Greening and Leedle, 1989) and of *Ruminococcus* and *Megasphaera* which are both known to produce butyrate (Claesson et al., 2012; Counotte et al., 1981).

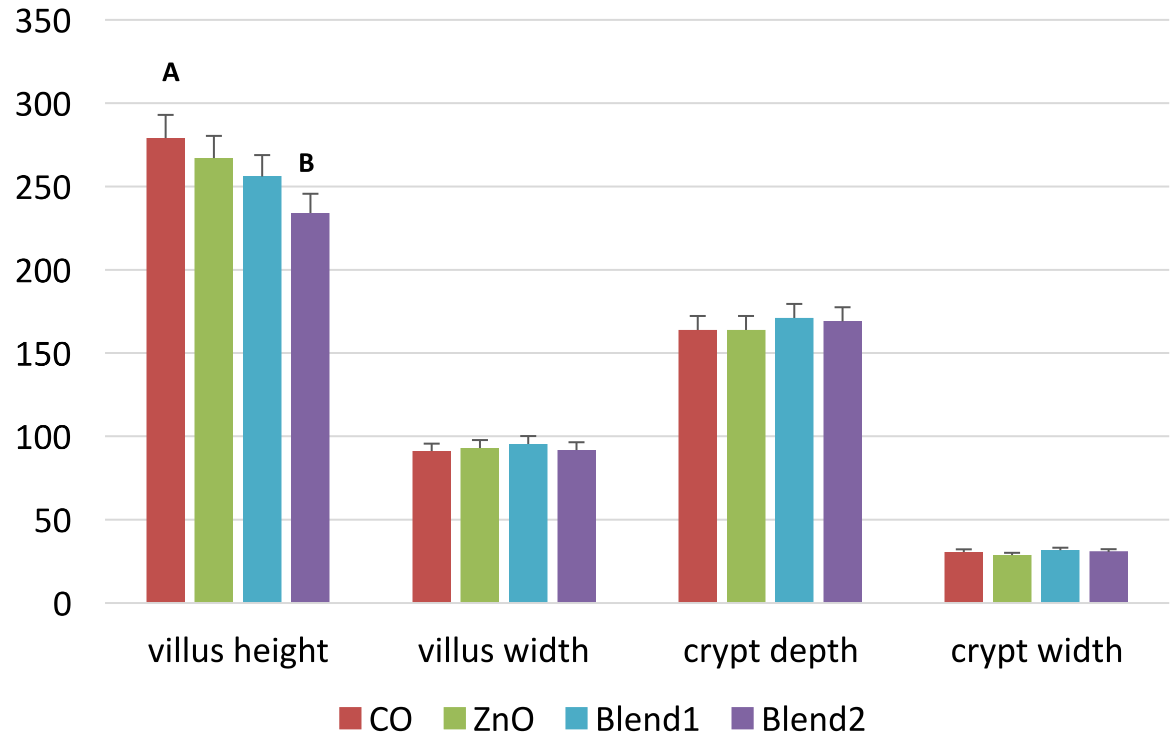
Silva et al. (2022) showed that the supplementation of phytogenic with a similar composition (essential oils: 41% garlic oil, 6% essential oil of cinnamic aldehyde, thymol, carvacrol and eugenol) of Blend1 to weaned piglets promoted the cecum abundance of *Megasphaera*.

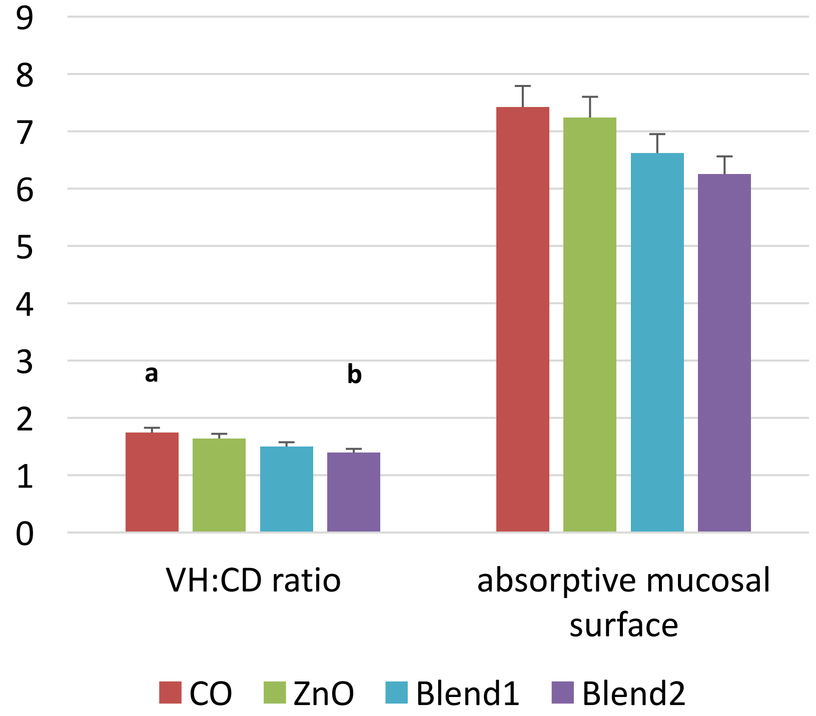
The Blend2 promoted the abundance of SCFAs producing bacteria including Christensenellaceae_R-7_group, Oscillospiraceae UCG-002 and Butyricicoccaceae UCG-008

da Silva, C.A et al., 2022. Plos one 17: e0279197; Greening, R.C. and Leedle, J,A., 1989. Archive in Microbiology 151:399–406. Claesson, M.J., et al., 2012. Nature 488:178-184; Counotte, G.H.M. et al., 1981. Applied and Environmental Microbiology 42:649-655.

Results: Intestinal morphology

Morphometric results at d35





No significant difference was observed between groups at d14

The Blend2 reduced the functional capacity of the small intestine which could also be associated with the limited FI of this group of pigs (Dong and Pluske 200).

Dong, G.Z, Pluske, J.R. 2007; Asian-Australasian Journal of Animal Sciences 20: 440-452

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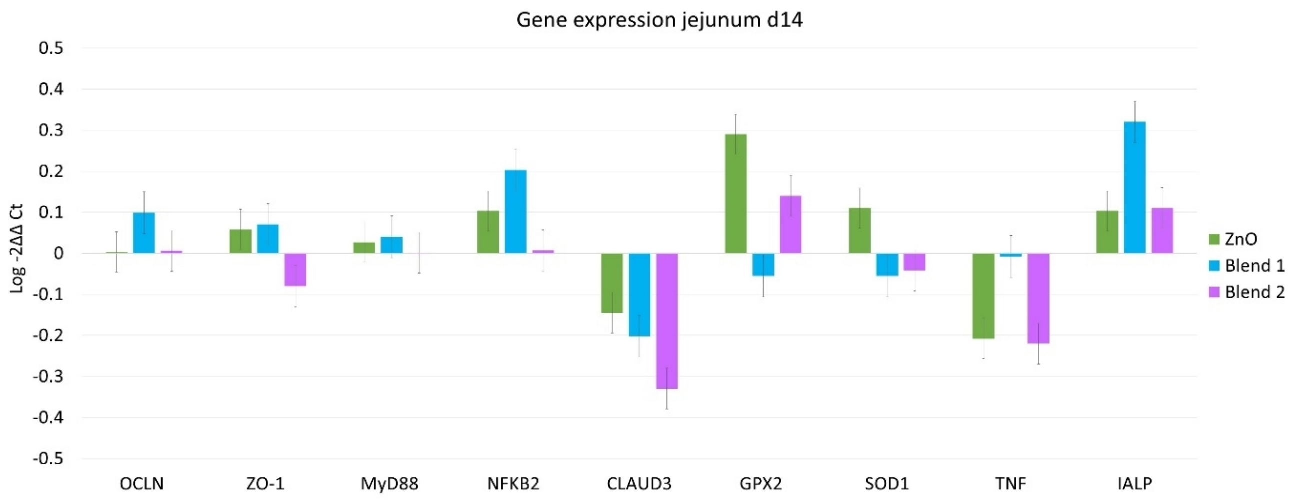
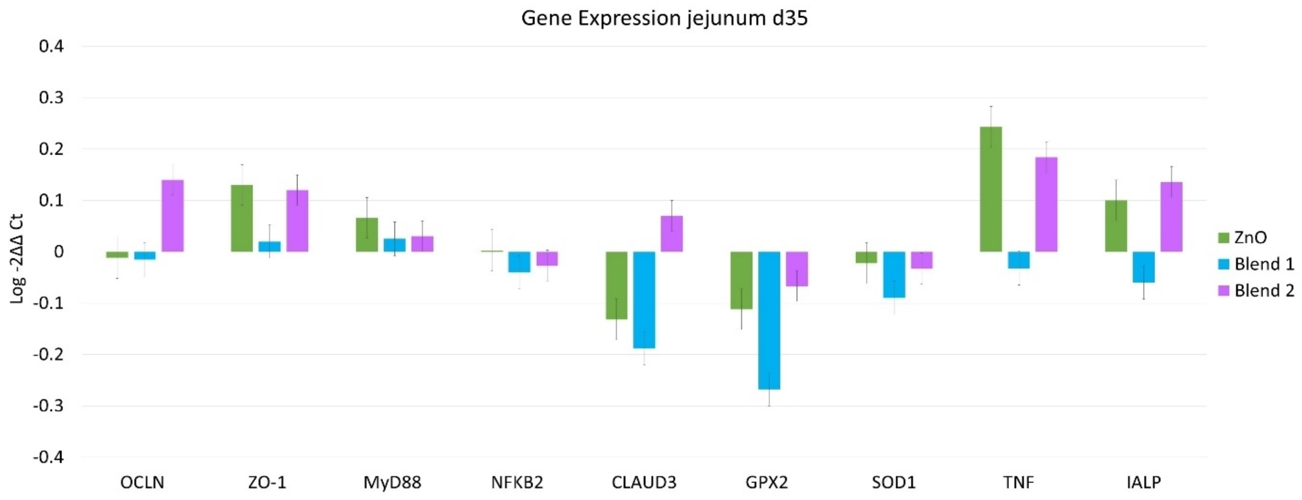
Results: Gene expression and blood metabolites





No significant effects were observed for the gene expression in the jejunum

No effect on the concentration of reactive oxygen metabolites in blood.

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Final considerations

- ✓ Using ZnO in healthy piglets modified the gut ecosystem, increasing the intestinal pH, reducing the taxa abundance and affecting the bacterial structure without improving the growth performance.

- ✓ Including natural and natural identical essential oils mixture in a post-weaning diet could be a potential strategy to maintain the gut health of post-weaning piglets, however, the efficacy of these blends varies according to their composition and dose of inclusion in the diet.

- ✓ The Blend 1 (cinnamaldehyde (naturally identical), cassia oil (titrated in cinnamaldehyde), aiowan essential oil (titrated in thymol) and essential oil of clove (titrated in eugenol)) allowed to modulate the gut microbiome promoting the abundance of SCFAs producing bacteria and reducing the one of haemolytic *E. coli*; therefore, it contributed in maintaining gut health in post-weaning piglets.

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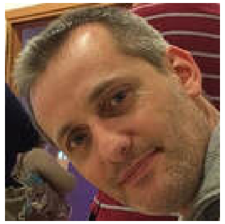

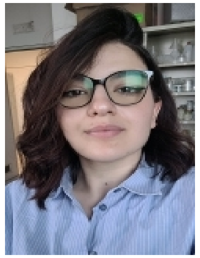

Thanks for your attention!

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