



ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA



Lyon, France - August 28th / September 1st, 2023

Effect of camelina cake doses as soybean meal substitutions on growth and gut health of piglets

D Luise ⁽¹⁾, ***F Correa*** ⁽¹⁾, ***S Virdis*** ⁽¹⁾, ***C Negrini*** ⁽¹⁾,
G Cestonaro ⁽²⁾, ***L Nataloni*** ⁽²⁾, ***G Titton*** ⁽²⁾, ***E Sattin*** ⁽³⁾, ***E***
Costanzo ⁽²⁾, ***P Trevisi*** ⁽¹⁾

(1) University of Bologna/ Department of Agricultural and Food Sciences, Bologna, Italy

(2) Cereal Docks S.p.A, Camisano Vicentino, VI, Italy

(3) Biomolecular Research Genomics Srl, Padova, PD, Italy

Introduction

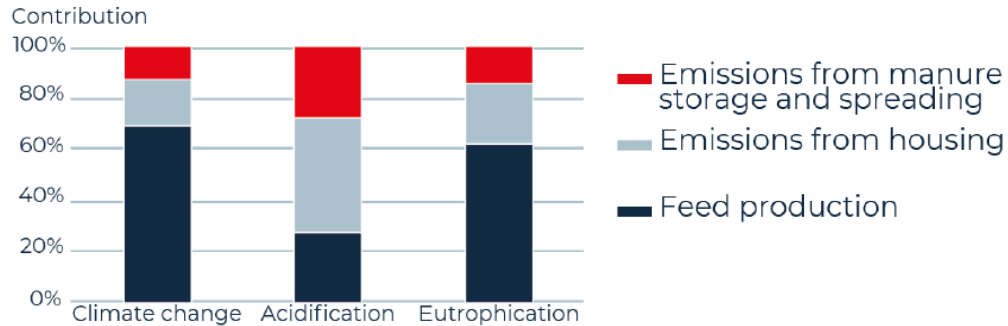


Table 3 Potential environmental impacts of producing 1 kg of each feed ingredient¹

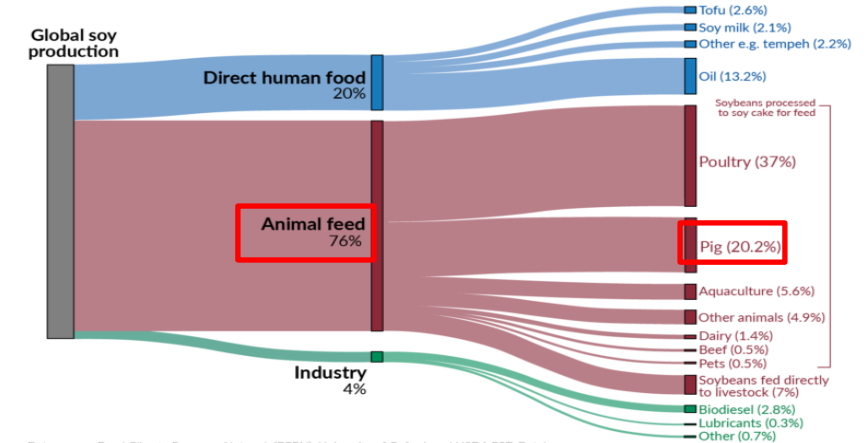
Feed ingredients	Climate change (g CO ₂ -eq)	Eutrophication (g PO ₄ -eq)	Acidification (g SO ₂ -eq)	Energy demand (MJ)	Land occupation (m ² -year)
Wheat					
Conventional	538	3.8	4.4	3.7	1.44
Organic	432	1.0	4.3	4.2	2.90
Maize	427	4.0	5.2	5.1	1.24
Barley					
Conventional	503	4.0	4.0	3.7	1.58
Organic	442	2.4	4.5	3.8	3.30
Peas	373	8.6	1.6	4.0	2.56
Rapeseed meal					
Conventional	456	3.3	3.8	4.1	1.01
Organic	319	0.5	3.2	3.6	2.10
Soybean meal					
Conventional	624	5.9	5.2	9.3	1.80
Organic	765	1.2	8.0	8.2	1.70
Monocalcium P	1202	14.9	30.8	18.4	0.32

¹Data for conventional feed ingredients was adapted from Mosnier *et al.* (2011). Data for organic ingredients are for Denmark (adapted from the LCA Food Database, 2007).

The World's Soy: is it used for Food, Fuel, or Animal Feed?

Shown is the allocation of global soy production to its end uses by weight. This is based on data from 2017 to 2019.

Our World in Data



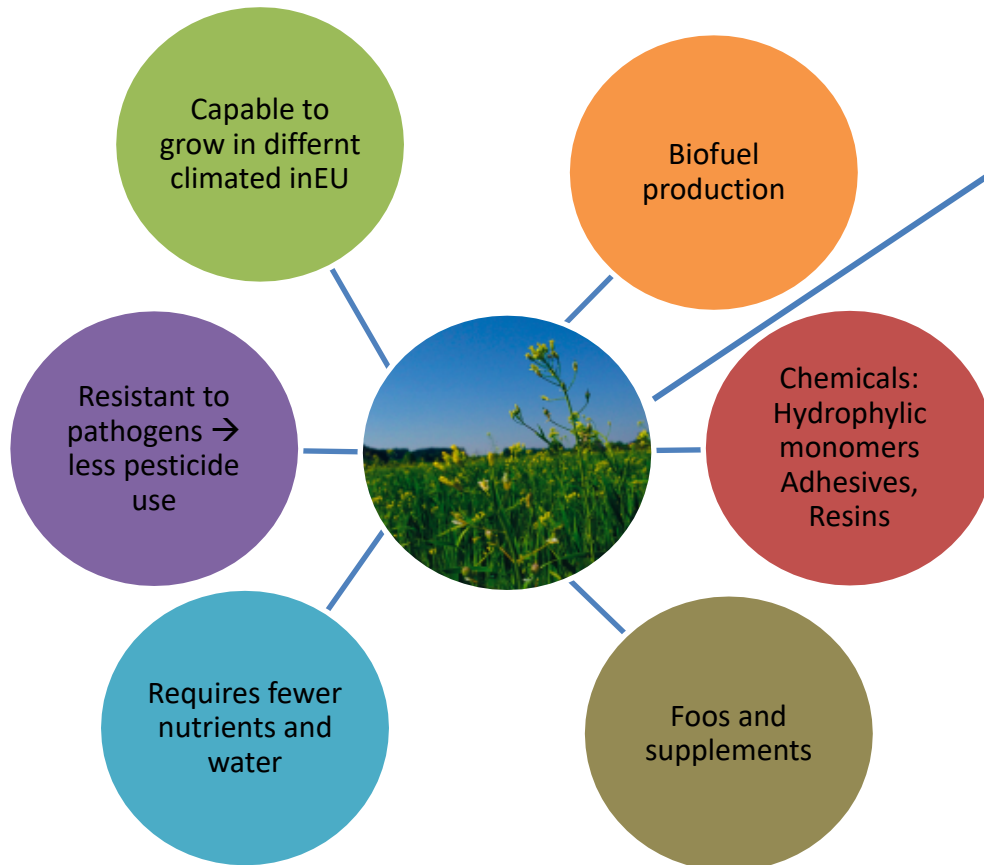
Data source: Food Climate Resource Network (FCRN), University of Oxford; and USDA PSD Database. OurWorldinData.org - Research and data to make progress against the world's largest problems. Licensed under CC-BY by the author Hannah Ritchie.

- The European Parliament has repeatedly called for the sources of animal feed to be diversified
- Reducing the EU's dependency on key imported agricultural products and inputs, in particular vegetal proteins for animal feed
- Improve agricultural supply chains' sustainability and circularity

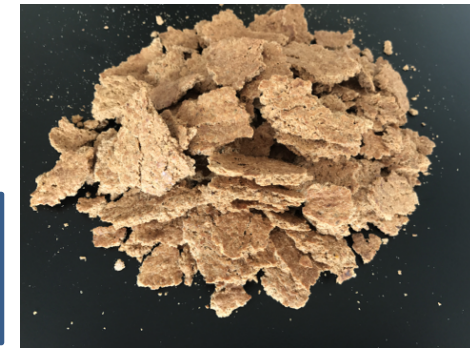


Introduction

Camelina [Camelina sativa (L.) Crantz], also called false flax, linseed dodder, or gold-of-pleasure is an oilseed crop in the Brassicaceae family



Animal feed



Crude protein (up to 30%-35%)
Residual oil content (from 10% to 30%)
Glucosinolates (34.4 to 36.3 mol/g)
Antitrypsin factor (12 to 28 TIU/mg)

Studies in pigs

- 6% camelina cake (CAM) reduced FI, ADG and BW during the first 28 days post-weaning (Smit et al., 2017).
- 10% of CAM reduced FI and ADG in fattening pigs (Li et al., 2021)



→ Low digestibility?

A study of Almeida et al (2013) showed that the digestibility of CAM is almost identical to that of rapeseed meal.

Hypothesis and aim

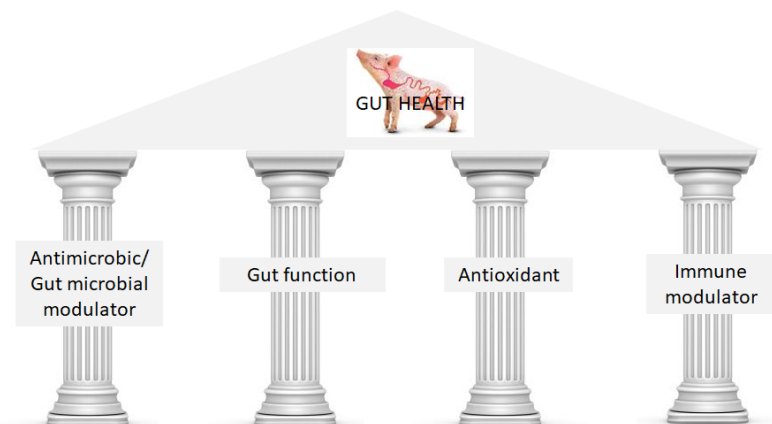
Including CAM in the swine diets can benefit swine production sustainability and reduce the EU dependence on imported soy

The decreasing sensitivity to anti-nutritional factors and improvement in the intestinal digestibility capacity with increasing age leads us to hypothesize the possibility of integrating CAM in pigs weighing 12-15 kg of live weight when the intestine has reached a good level of maturity.

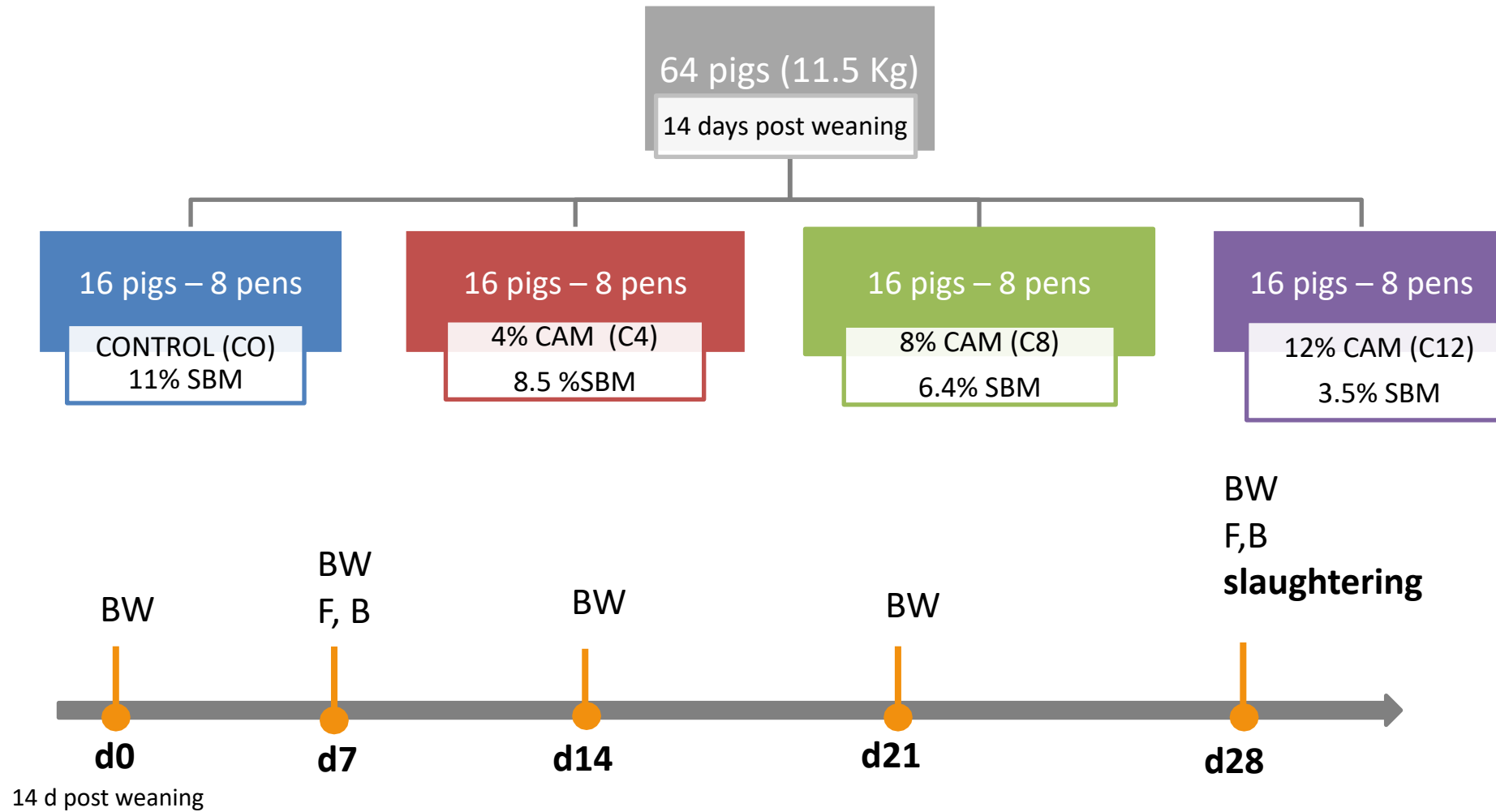
Few studies are available and the correct amount of CAM inclusion in the diet of growing pigs is still poorly understood.



This study aimed to evaluate the effect of different doses of CAM in substitution of soybean meal on growth performance and gut health of weaned pigs



Material e methods



BW: Body weigh; F: Faeces; B: Blood



Material e methods

Analysis	Tissue	Time	N samples
NGS microbiota (v3-v4 regions 16S rRNA)	Faeces	d7, d28	128
Polyamines	Faeces	d7, d28	128
pH	Jejunum, cecum and colon	d28	64
ROMs	Blood	d7, d28	128
T4 hormon	Blood	d28	64
Organs weight	Small intestine, colon, kidneys, liver and spleen	d28	64
Genes expression: ZO-1, OCLN, GPX2, Myd88, TNF	Jejunum	d28	64
Intestinal morphology	Jejunum	d28	64

Statistical analysis

Data were analyzed using a linear mixed model and ANOVA procedure considering the diet (C0, C4, C8, C12) as a fixed factor and the pen and litter as random factors.

Linear and quadratic polynomial contrasts for the diet were carried out.

P-values ≤ 0.05 were considered statistically significant, while P-values ≤ 0.1 were considered a trend of significance.



Results: Body weight and average daily gain

Item	Diet ¹				SEM	P-value Diet	Effect	
	CO	C4	C8	C12			Linear	Quadratic
Body weight, g								
d0	11562	11563	11563	11563	530	1.00	1.00	1.00
d7	14918	14119	14186	13922	595	0.44	0.16	0.56
d14	19133	18376	18425	17728	720	0.41	0.13	0.96
d21	24620	23431	23693	22325	907	0.19	0.06	0.91
d28	29669	27922	28871	26539	1043	0.10	0.05	0.76
Average daily gain g/d								
d0 - d7	480	366	376	338	24.2	<0.0001	0.0004	0.11
d7-d14	604	610	607	545	35.4	0.49	0.26	0.33
d0 - d14	542	488	491	441	26.7	0.04	0.01	0.93
d14 - d21	784	722	752	656	40	0.12	0.05	0.66
d0 - d21	623	566	578	513	27	0.03	0.01	0.87
d21 - d28	719	640	738	623	44.4	0.17	0.34	0.69
d14 - d28	751	681	745	628	37.5	0.06	0.08	0.54
d7 - d28	703	657	699	601	30.9	0.07	0.07	0.39
d0 - d28	647	585	619	535	25.9	0.02	0.01	0.69

In accordance with previous studies carried out on weaning (Smit 2017) and finishing (Li et al., 2021) pigs, the inclusion of CAM can linearly reduce the ADG of pigs. In the present study, the effect was mainly observed in the first week of administration (3rd week post-weaning).



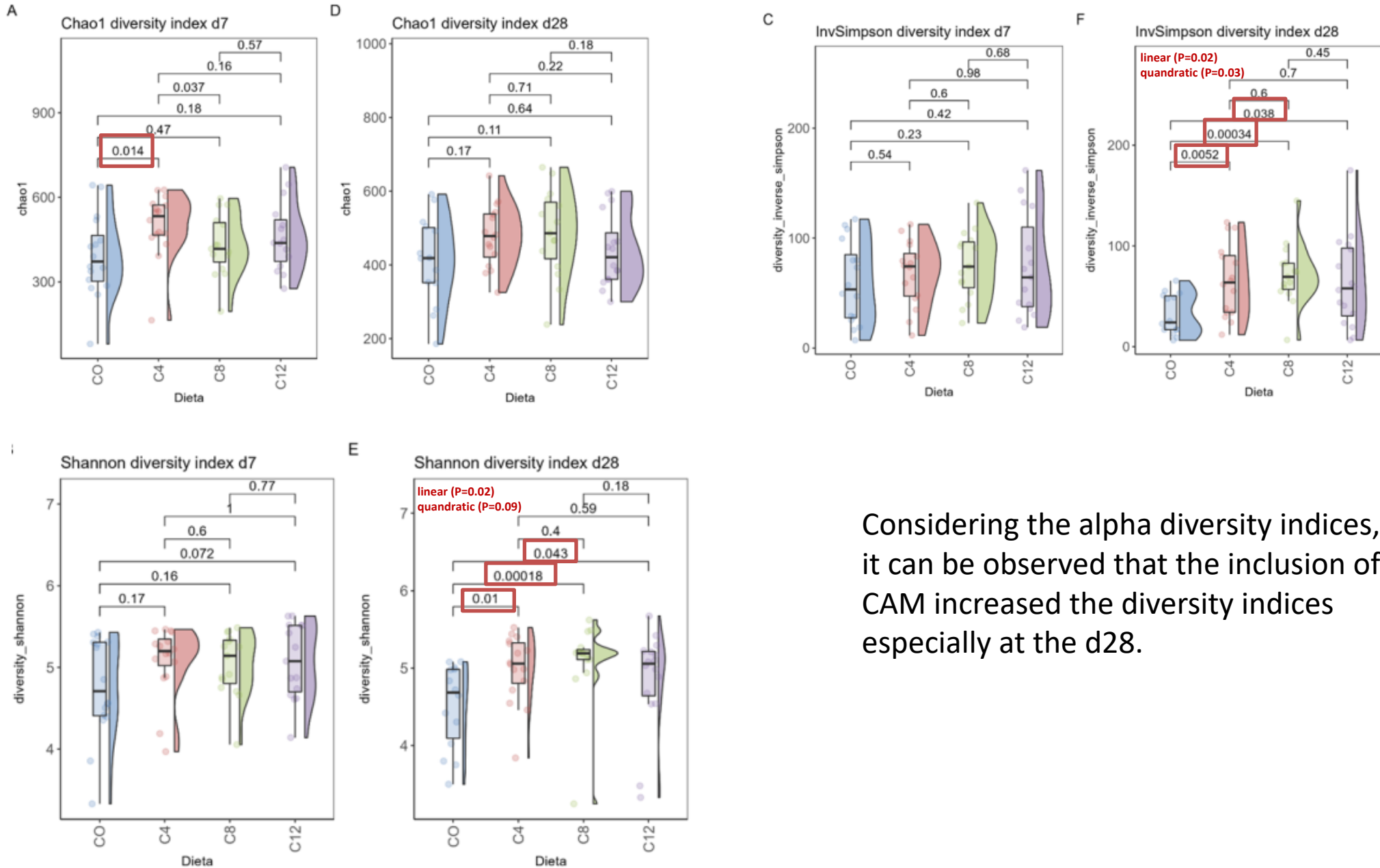
Results: feed intake and feed conversion ratio

Item	Diet ¹				SEM	P-value Diet	Effect	
	CO	C4	C8	C12			Linear	Quadratic
Feed intake g/d								
d0 - d7	674	598	624	579	27.50	0.10	0.04	0.58
d7-d14	941	941	929	856	49.80	0.56	0.23	0.47
d0 - d14	808	769	777	718	34.70	0.34	0.10	0.77
d14 - d21	1183	1143	1159	1044	53.10	0.29	0.10	0.49
d0 - d21	933	894	904	826	37.40	0.25	0.08	0.61
d21 - d28	1300	1164	1249	1112	72.90	0.28	0.15	1.00
d14 - d28	1241	1154	1204	1078	59.60	0.26	0.11	0.75
d7 - d28	1141	1083	1112	1004	51.40	0.29	0.11	0.64
d0 - d28	1025	961	990	898	42.30	0.21	0.07	0.73
FCR								
d0 - d7	1.42	1.65	1.66	1.74	0.07	0.02	0.004	0.26
d7-d14	1.56	1.55	1.53	1.57	0.04	0.93	0.89	0.61
d0 - d14	1.49	1.58	1.58	1.63	0.04	0.12	0.02	0.72
d14 - d21	1.52	1.59	1.54	1.60	0.04	0.42	0.29	0.92
d0 - d21	1.50	1.58	1.56	1.61	0.03	0.13	0.04	0.66
d21 - d28	1.82	1.83	1.71	1.84	0.07	0.54	0.83	0.44
d14 - d28	1.66	1.70	1.62	1.72	0.05	0.42	0.64	0.47
d7 - d28	1.63	1.65	1.59	1.66	0.04	0.55	0.81	0.50
d0 - d28	1.59	1.64	1.60	1.67	0.03	0.31	0.20	0.80

In agreement with ADG data, the inclusion of CAM tended to reduce feed intake and increase the FCR of pigs in the first week of administration. However, this effect was not maintained in the following weeks.



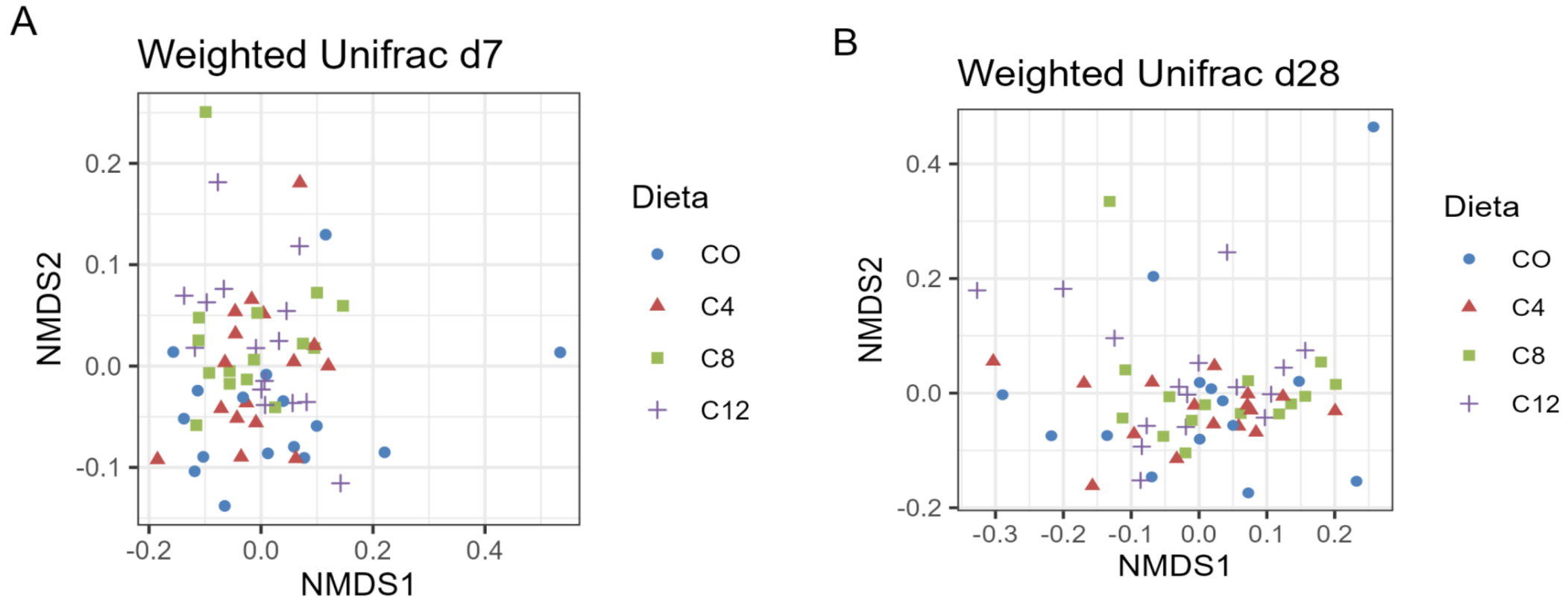
Results: microbiota



Considering the alpha diversity indices, it can be observed that the inclusion of CAM increased the diversity indices especially at the d28.



Results: microbiota



For Beta diversity, the graphs show that samples from the different diets do not form separate clusters, both at d7 and d28, indicating that the general microbial structure between the experimental diets is similar. This is also evidenced by the Adonis statistical test which was not significant either at d7 ($R^2=0.06$, $P=0.21$) or at d28 ($R^2=0.05$, $P=0.36$).



Results: microbiota

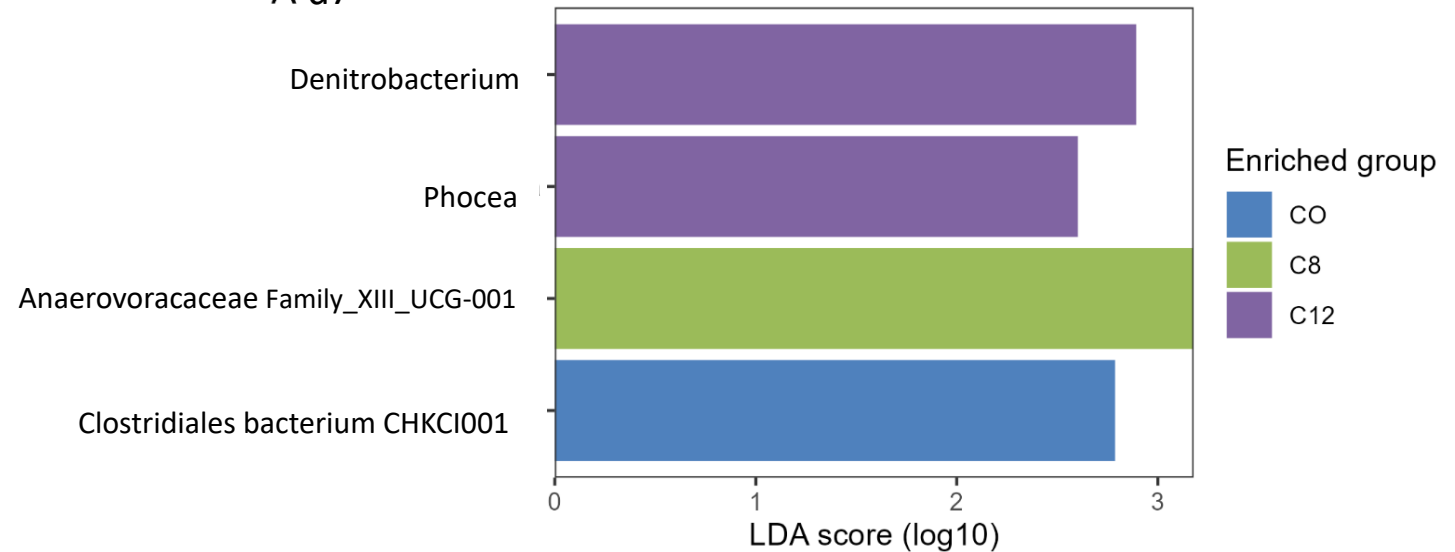
Genera in the family Butyricocccaceae are generally considered beneficial for their production of butyric acid.

The genera belonging to the family Erysipelatoclostridiaceae the data are still unclear. Indeed, it seems that an excess in the abundance of some genera of this family is associated with intestinal disorders in humans.

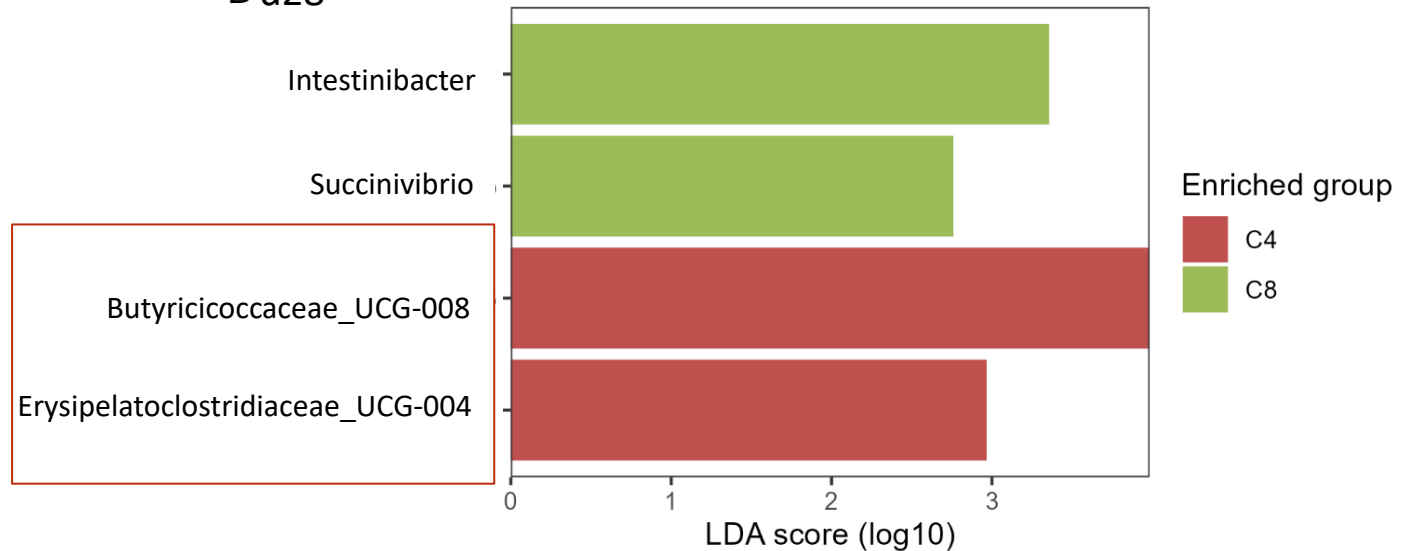
However, in pigs, an increase in genera belonging to the Erysipelatoclostridiaceae family was observed following the administration of probiotics (Luise et al., 2021) and free amino acids (Correa et al., 2022).



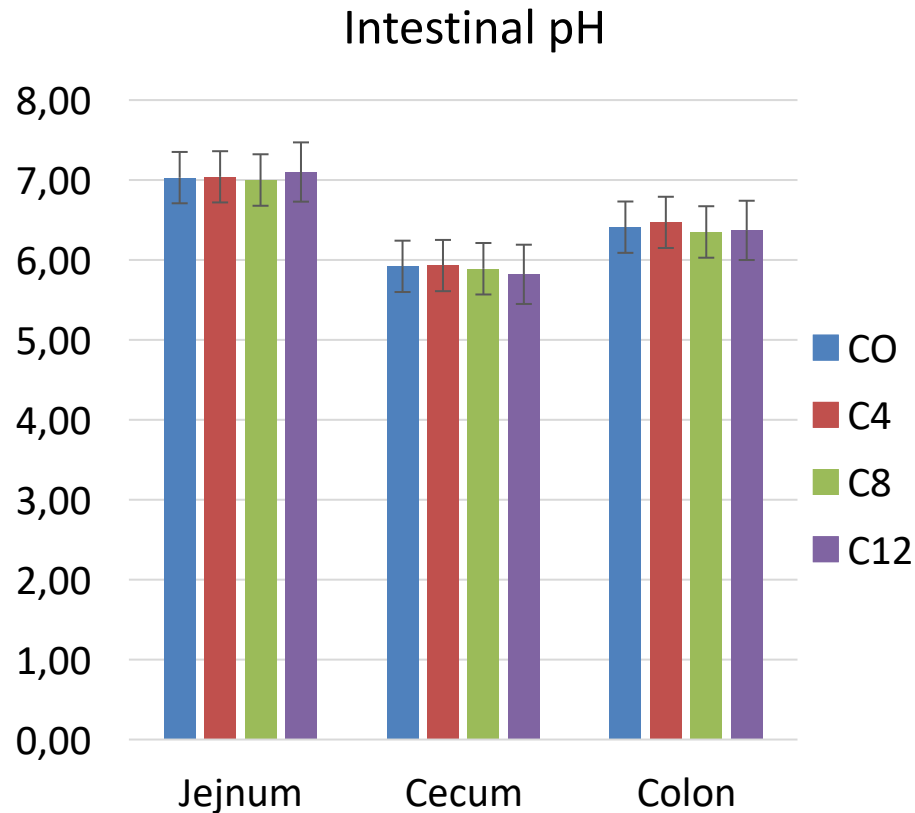
A d7



B d28



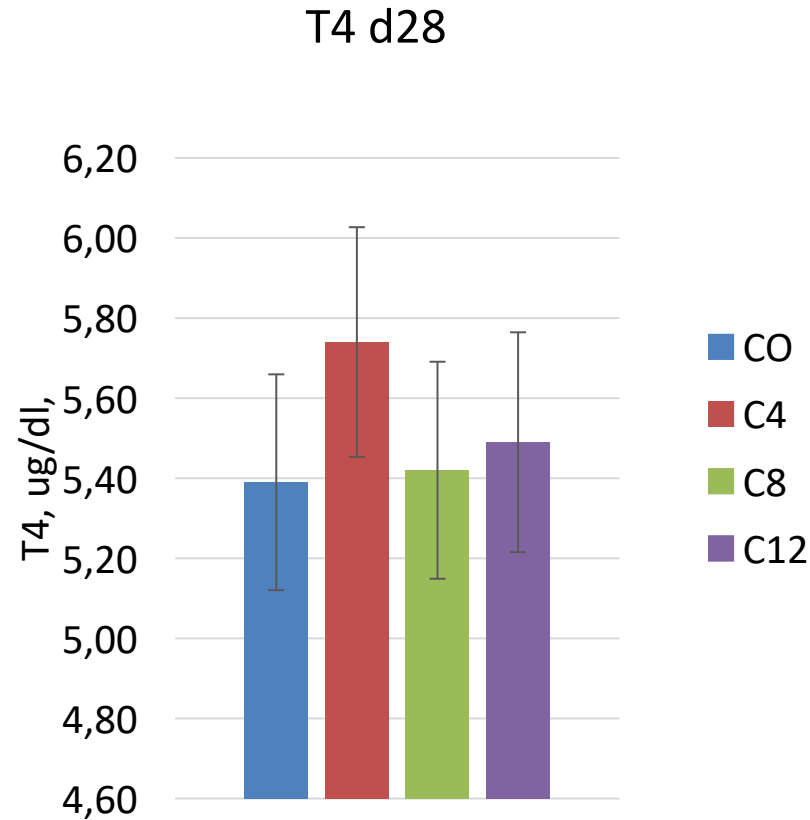
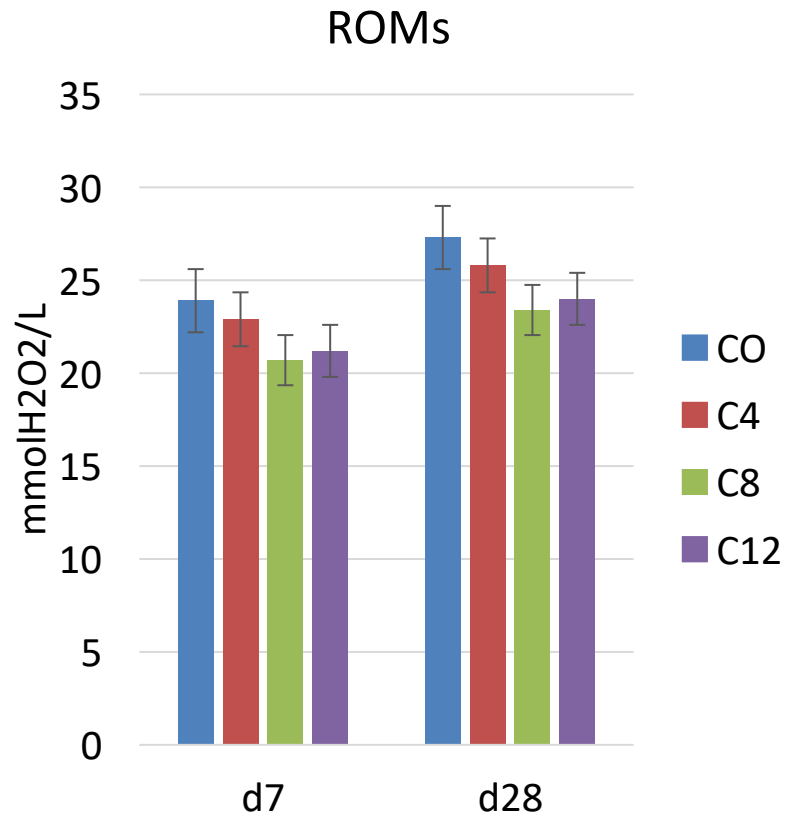
Results: pH and microbial fermentation data



Polyamines faeces, nmol/g	Diet*				SEM	P			
	CO	C4	C8	C12		Diet	Linear	Quadratic	
d7									
Putrescine	112.8	126.1	73.8	80.6	24.3	0.40	0.20	0.89	
Cadaverine	174	90.3	110.4	828.3	20.2	0.37	0.22	0.26	
Spermidine	378	331	312	387	41.1	0.50	0.96	0.14	
Spermine	28.8	37.2	22.6	19.6	23.5	0.22	0.15	0.38	
d28									
Putrescine	85	75.1	72.2	75.4	23.3	0.83	0.49	0.53	
Cadaverine	32.4	40.4	54.1	38.2	19.3	0.88	0.72	0.54	
Spermidine	236	175	207	208	11.8	0.11	0.49	0.09	
Spermine	18.8	16.8	16.3	18.4	2.50	0.86	0.89	0.41	



Risultati: blood parameters

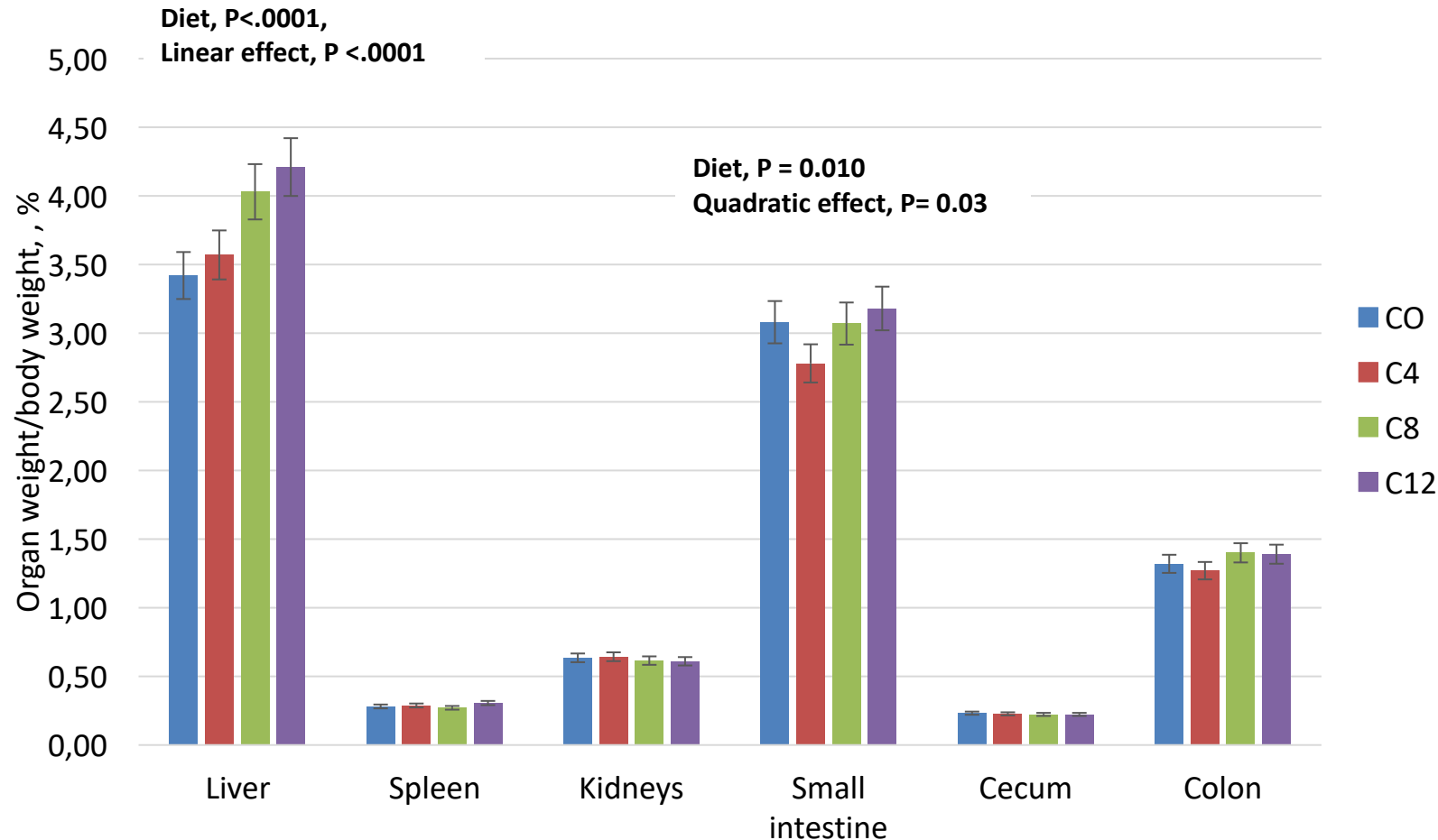


The inclusion of CAM did not disturb the oxidative state of the pigs as well as the functionality of the thyroid which could have been altered by the presence of glucosinolates in the CAM diets



Results: Organs weight

Organs weight d28



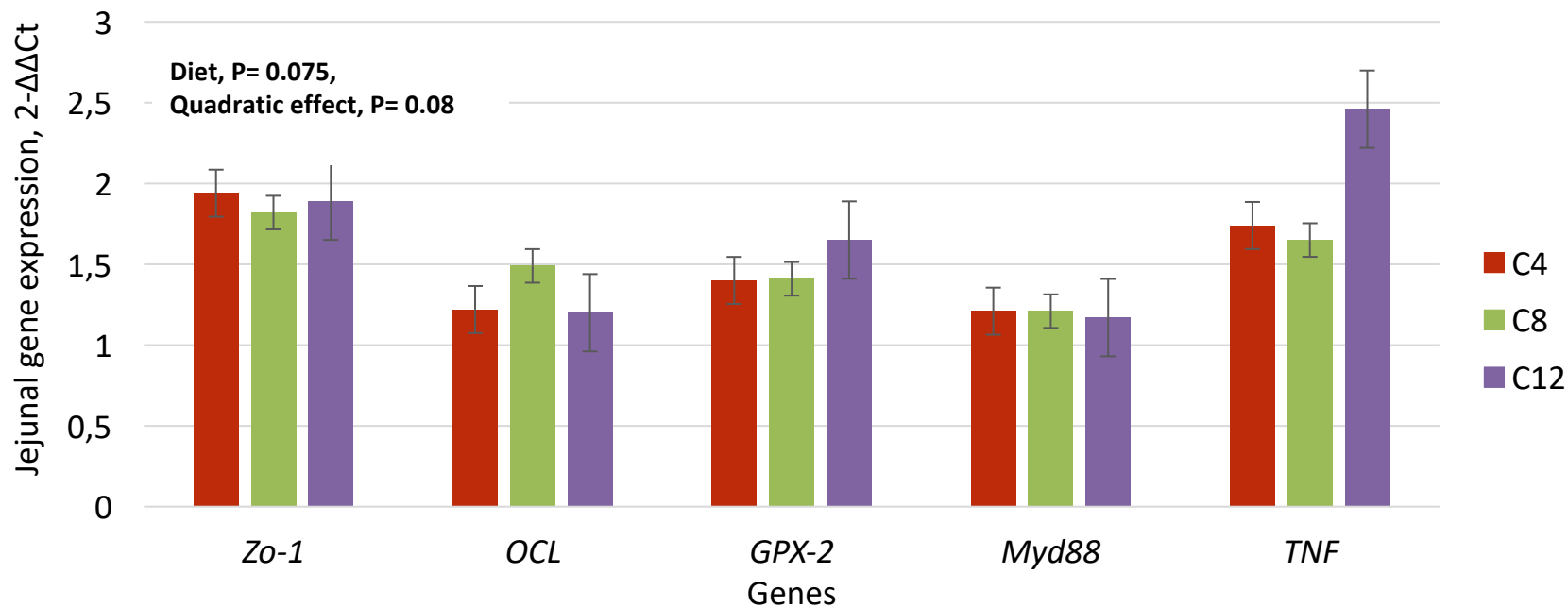
The increase in liver weight following the inclusion of CAM was also observed in previous studies (Smit 2017; Meadus et al. 2014).

A possible reason is the high presence of antinutritional factors in CAM which explains the increased activity of the liver (Meadus et al. 2014)

Risultati: jejunal morphology and gene expression parameters

Jejunal morphological parameters, d28	Diet				SEM	P- value Diet	Effect	
	C0	C4	C8	C12			Linar	Quadratic
Villus height, μm	276	263	252	280	12	0.28	0.95	0.08
Villus width, μm	101.3	96.7	92.2	96.5	4.6	0.27	0.20	0.18
Crypt width, μm	172	154	170	170	9.3	0.14	0.70	0.15
Crypt depth, μm	41.4	37.8	39.1	19.4	2.38	0.66	0.59	0.52
VH:CD ratio	1.68	1.75	1.52	1.69	0.11	0.19	0.56	0.50
M index	6.18	6.16	5.98	6.47	0.28	0.65	0.57	0.37

Jejunum gene expression, d28



The *Zo-1* gene encodes a member of the membrane-associated guanylate kinase protein family that acts as a tight junction protein, involved in the passage of ions and macromolecules in the intestine.

Data suggesting that CAM can slightly modulate the expression of genes involved in the intestinal barrier.



Discussion

- The inclusion of CAM in pig diets from three to eight weeks post-weaning did not affect the intestinal health their oxidative status of pigs
- However, its inclusion reduced the animals' ADG, especially during the first week of administration, which effect was not completely recovered in the following weeks.
- The reduction in ADG is not attributable to a decrease in FI, for which only a trend was recorded in the first week, but to a reduction in feed conversion capacity
- The high presence of antinutritional factors did not compromise the synthesis of thyroid hormone which is involved in the metabolic processes necessary for growth, however, they may have contributed to increase in the metabolic activity and in the energy required by the liver, whose weight increased with the increasing of CAM in the diet.

Take home message

- Overall our results suggest to include CAM in the swine diet after three weeks post-weaning to maintain growth performance.
- To favour the inclusion of CAM in swine diets it is suggested to treat the product through technological processes to improve its nutritional composition and digestibility.



Thanks for your attention

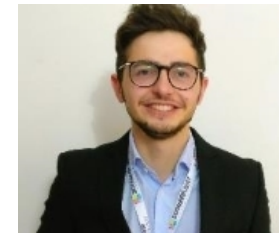
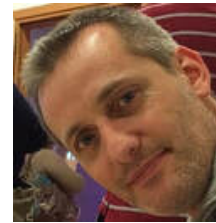
D Luise ⁽¹⁾, ***F Correa*** ⁽¹⁾, ***S Virdis*** ⁽¹⁾, ***C Negrini*** ⁽¹⁾, ***G Cestonaro*** ⁽²⁾,
L Nataloni ⁽²⁾, ***G Titton*** ⁽²⁾, ***E Sattin*** ⁽³⁾, ***E Costanzo*** ⁽²⁾, ***P Trevisi*** ⁽¹⁾

diana.luise2@unibo.it

⁽¹⁾ *University of Bologna/ Department of Agricultural and Food Sciences, Bologna, Italy*

⁽²⁾ *Cereal Docks S.p.A, Camisano Vicentino, VI, Italy*

⁽³⁾ *Biomolecular Research Genomics Srl, Padova, PD, Italy*



FA&AF project (ID 10288429), POR FESR 2014-2020 Action 1.1.4, Veneto Region, Italy.

European Union Next-GenerationEU (PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) – MISSIONE 4 COMPONENTE 531 2, INVESTIMENTO 1.4 – D.D.532 1032 17/06/2022, CN00000022)



Diet composition

Items	Diet				Items	Diet			
	CO	C4	C8	C12		CO	C4	C8	C12
Ingredients, %					Calculated chemical composition, %				
Barley	19.7	19	17.7	19	Dry matter	89.83	89.93	90.04	90.16
Flaked barley and corn	15	15	15	15	Humidity	10.17	10.07	9.96	9.84
Corn	14	14	14	12	Crude Protein	16.45	16.47	16.63	16.57
Soybean meal	11	8.5	6.4	3.5	Fats	5.48	5.29	5.58	5.51
Camelina cake	-	4	8	12	Fiber	3.44	3.72	4.03	4.42
Wheat flour	10	10	10	10	Ashes	4.38	4.43	4.52	4.60
Wheat	8.2	8	8	8	NDF	14.39	14.92	15.41	16.29
Bran	6	5	5	5	ADF	4.88	5.40	5.95	6.56
Lactose	3	3	3	3	ADL	0.82	1.03	1.25	1.49
Fish meal	2	2	2	2	Starch	40.56	40.29	39.21	38.57
Soybean oil	2	2	1.5	1.5	ED Kcal/kg	3378.1	3358.4	3358.0	3325.2
Soy protein concentrate (63% CP; Soycomil R)	2	2	2	2	EM Kcal/kg	3241.4	3222.9	3222.8	3191.2
Beet pulp	1.5	1.5	1.5	1.5	Lys SID	1.13	1.08	1.04	0.98
Oat bran	1	1	1	1	Met SID	0.44	0.46	0.47	0.44
Coconut oil	1	1	1	1	Trp SID	0.23	0.22	0.20	0.19
Bicalcium Phosphate	1	1	1	1	Thr SID	0.74	0.70	0.66	0.60
L-LysineHCL	0.58	0.61	0.63	0.65	Met+Cist SID	0.70	0.73	0.75	0.74
Sodium Chloride	0.4	0.4	0.4	0.4	Val SID	0.76	0.71	0.66	0.61
Enzymes Premix	0.3	0.4	0.4	0.4	Ile SID	0.59	0.54	0.50	0.44
Calcium Formate	0.3	0.3	0.4	0.3	Leu SID	1.09	1.01	0.93	0.83
L-Threonine	0.25	0.26	0.26	0.24	Ist SID	0.37	0.34	0.32	0.29
Amino Acid premix:Val , Ile, Leu, His	0.25	0.25	0.25	0.25	Arg SID	0.83	0.75	0.67	0.58
DL-Methionine	0.21	0.21	0.21	0.18	Ca	0.69	0.70	0.71	0.72
Flavoring	0.09	0.09	0.09	0.09	P	0.71	0.69	0.67	0.66
L-Triptophan	0.045	0.05	0.05	0.05	Analysed Glucosinolates mmol/kg Dry matter	not detectable	not detectable	1.53	4.17



CAM and diet analysed composition

Items	CAM	CO	C4	C8	C12
Crude protein	28.40	17.48	16.74	17.06	16.89
Fat	4.64	5.71	5.78	5.49	5.53
Cellulose		4.21	4.78	4.31	5.02
ADF	15.00	4.00	5.50	5.40	6.10
NDF	21.30	16.80	18.70	17.60	17.90
ADL	3.50	2.10	0.90	1.40	1.30
Ashes	9.45	5.03	5.16	5.15	5.47
Lysine	1.31	1.47	1.45	1.36	1.54
Methionine	0.48	0.42	0.42	0.39	0.46
Cysteine (determined as cysteic acid)	0.56	0.29	0.27	0.28	0.28
Aspartic acid	2.30	1.39	1.28	1.25	1.24
Threonine	1.14	0.81	0.82	0.74	0.86
Serine	1.23	0.73	0.69	0.69	0.69
Glutamic acid	4.74	3.27	3.14	3.13	3.13
Proline	1.48	1.10	1.09	1.08	1.08
Glycine	1.52	0.71	0.69	0.70	0.72
Alanine	1.29	0.76	0.73	0.73	0.73
Valine	1.45	0.89	0.90	0.97	1.07
Isoleucine	1.08	0.70	0.68	0.69	0.72
Leucine	1.83	1.27	1.23	1.26	1.31
Tyrosine	0.72	0.49	0.45	0.49	0.47
Phenylalanine	1.17	0.88	0.76	0.79	0.81
Histidine	0.68	0.45	0.45	0.46	0.50
Arginine	2.24	0.95	0.91	0.91	0.93
Tryptophan	0.32	0.23	0.24	0.24	0.24
Total amino acids	25.54	16.81	16.20	16.16	16.78

