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Feeding additives to replace the use of therapeutic doses of ZnO without rising antibiotic use in post-weaning piglets

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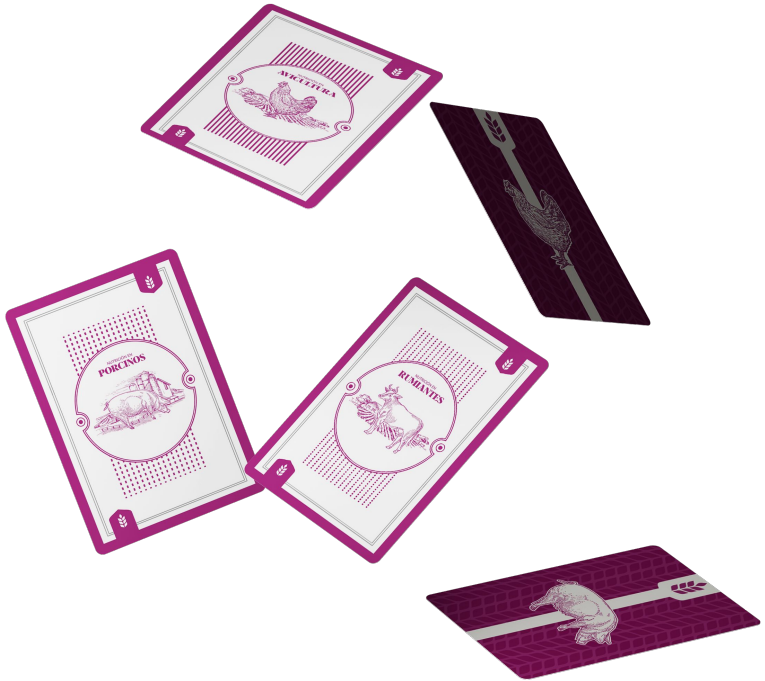
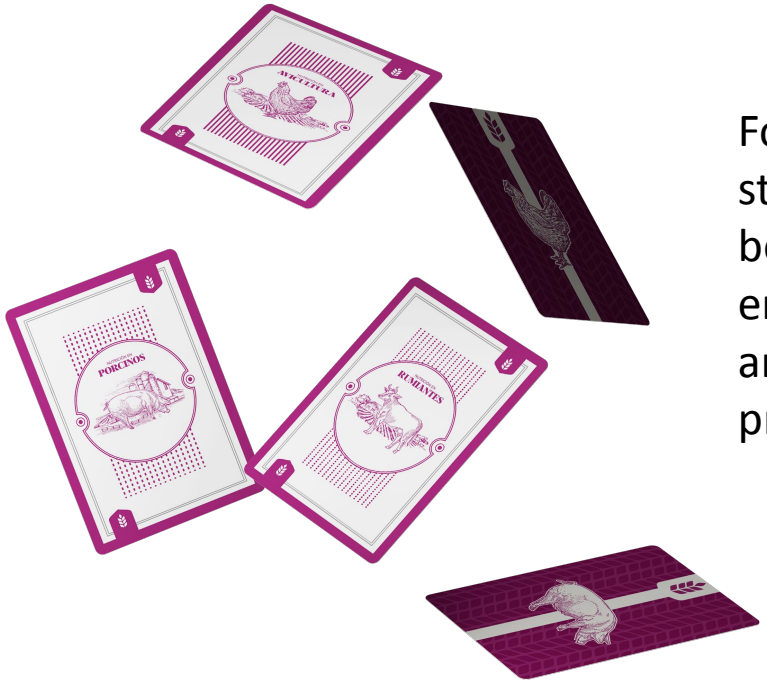
Structure of the presentation

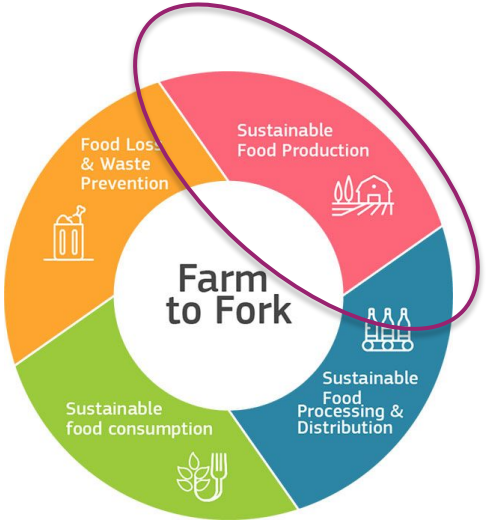
- Introduction of the EuropeanStateregy to reduce the spread of antibiotic resistance relate to livestocks.
- Role and modes of action of antibiotics and terapeutic ZnO.
- Concept of gut health.
- Feeding additives and nutrients to maintain gut health:
    - **Organic acids, short and medium fatty acids**
    - **Tannins**
    - **Probiotics**
    - **Enzymes and vitamins**
    - **Amino acids**



European Livestock production



Following the European strategy, pig production must be economically and environmentally sustainable and at the same time maintain product quality and safety.

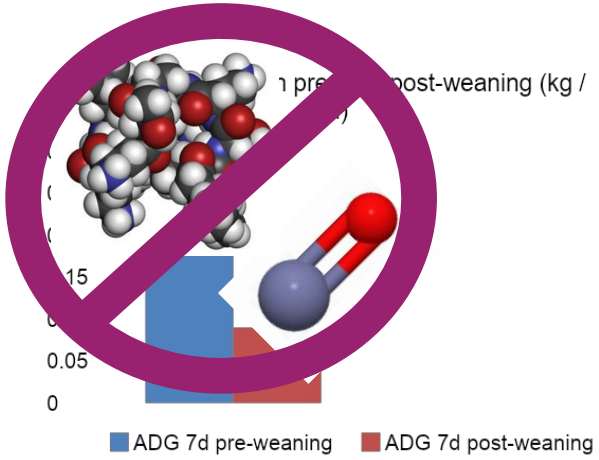


Effect of early weaning on piglet health and performance

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

Weaning period

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



↓ Feed intake

↓intestinal eubiosis

↑ Diarrhea, mortality
↓ Performance

DYSBIOSIS

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Evolution of European regulatory framework on antibiotics and zinc

Communication from the Commission to the European Parliament and the Council: Action plan against the rising threats from Antimicrobial Resistance
Published 15th September 2011

COMMISSION NOTICE Guidelines for the prudent use of antimicrobials in **veterinary medicine** (2015/C 299/04)
Published 11th September 2015

June 2017, the European Commission adopted the EU One Health Action Plan against AMR

Withdrawal of the authorization for veterinary products containing **Zinc Oxide** for oral administration in food producing animals
Enter into force from 25th June 2022

UE Reg2019/6 (11th December 2018)
New regulation for the veterinary medicine, abrogation of the EU directive 2001/82/CE
Enter into force from 28th January 2022

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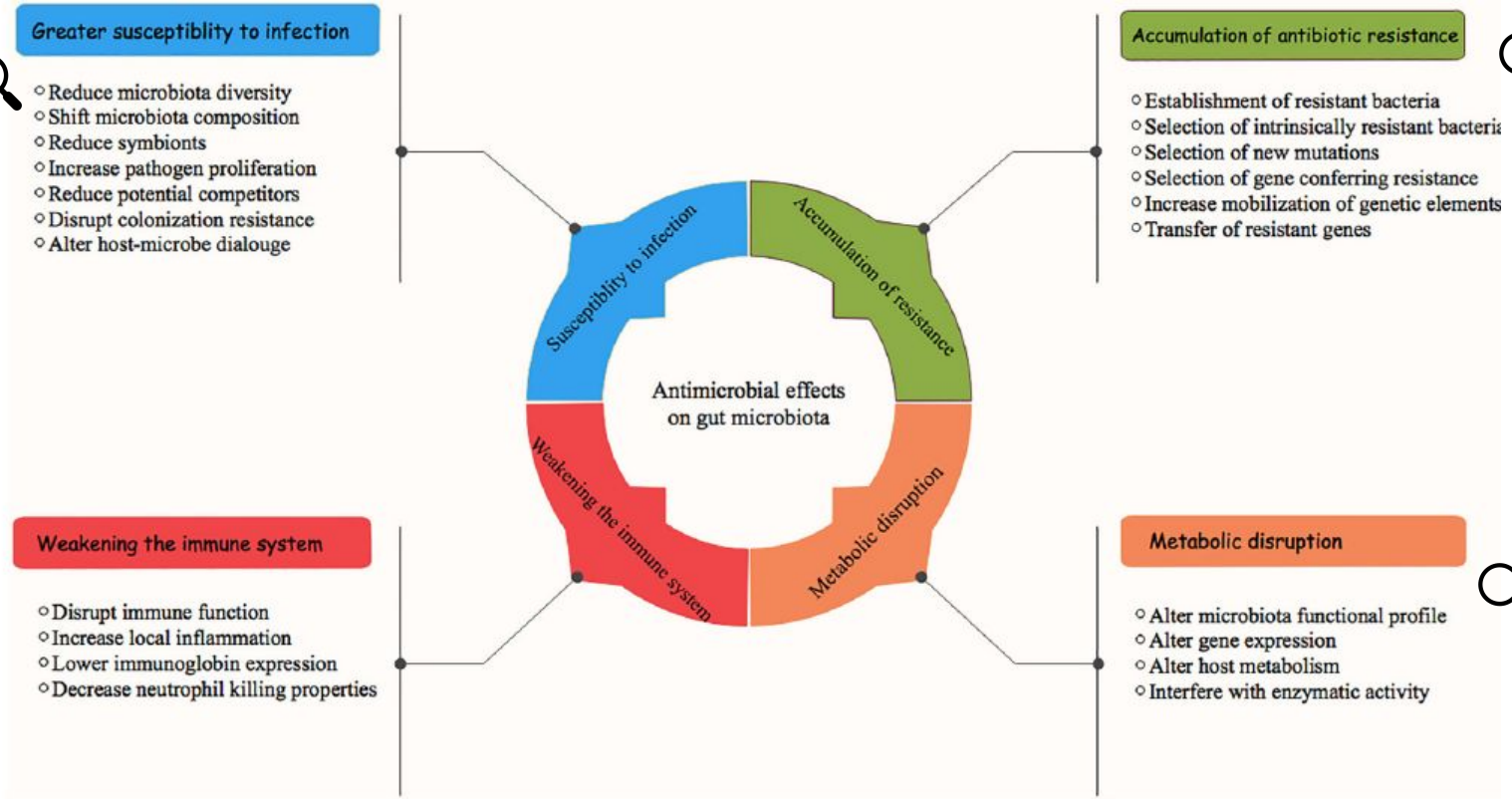
Effects of antimicrobial on gut health and consequences



Zeineldin et al.

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Effect of antibiotics on gut microbiome of pigs

N. pigs	Age	Samples type	Antimicrobial class	Effects			Ref
				Alpha	Beta	Taxa	
16 pigles	at birth	Faeces	Ceftiofur	=	=	> *Enterobacteriaceae, Coriobacteriaceae* and *Bifidobacteriaceae; < Christensenellaceae* and Clostridiales	*Ruczizka et al.*, 2020
6 pigles	3-19 weeks	Faeces	Tylosin	=	Separationto CO using Unifrac distance	< *Fibrobacteres*; > Coprococcus at 3 weeks. < *Streptococcus* and *Coprococcus* and >*Fibrobacter* at weeks 9; >*Coprococcus* and *Akkermansia* at weeks 19	*Holman and Chénier*, 2014
			Chlortetracycline	=	Separation to CO using Unifrac distance	< *Lactobcillus* > SMB53 at weeks 9	
12 pigets	12 weeks	ileum and colon	Chlortetracycline, sulfamethazine and penicillin	-	Separation to CO	< *Streptococcus; <Helicobacter, Turicibacter* and *Treponema* (colon); > *Escherichia, Lachnobacterium, Salsuginibacillus spp, Anaeroplasma* and *Para-prevotella spp.* in(colon)	*Looft et al.*, 2014a
6 piglets	12 weeks	Faeces	Carbadox (quinoxaline)	< during the fist week but than = to CO	Separation to CO during the fist week, but then =	> *Prevotella, Roseburia, Faecalibacterium*,and *Asteroleplasma*	*Looft et al*. 2014b
24 pregnant sows	-	Faeces	Mixture of: lincomycin, chlortetracycline, and amoxicillin.	-	-	<*Escherichia/Shigella* and *Streptococcus* but = after 12 days; > *Treponema, Enterococcus,* and *Staphylococcus* populations but = afetr 6 days	*Sun et al.*, 2014
4 piglets	28 days	Faeces	Chlortetracycline	-	=	=	*Poole et al.*, 2013

The alpha diversity indices are not the main target of the antibiotics

Beta diversity is always affected by antibiotics, nevertheless the type and doses

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Mechanisms of action of the pharmacological dose of ZnO

Zinc Oxide

- Limit the adhesion of ETEC
- Synthesis of > 300 enzymes
- Minimize intestinal unbalance
- Reduce incidence of diarrhea
- Improve growth performance
- Enhance gut health

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Mechanism of action of the pharmacological dose of ZnO: markers for gut integrity and inflammation

Trevisi et al. Meta-analysis approach on ZnO. Data not published

ITEM	N. studies	LZn	M1Zn	M2Zn	HZn	SEM	P-value		
							Zn level classes	Basal Zn	Reference
ZO-1	3	104.14[b]	-	-	144.75[a]	4.93	**0.01**	0.62	0.4
OCL	2	103.89	-	-	133.73	12.2	**0.06**	0.64	0.3
IL-1β	5	94.68	121.95		74.8	17.54	0.33	0.7	0.37
TNF-α	6	100.68	-	102.72	113.5	5.76	**0.09**	0.24	0.49
IFN-γ	6	97.15[a]	-	-	77.09[b]	4.99	**0.01**	0.29	0.37
TGF-β	4	99.46	100.59		123.43	8.47	0.15	0.08	-

LZn: 0 ppm to 200 ppm; M1Zn:201 ppm to 630 ppm; M2Zn: 631ppm to 1600 ppm; HZn from 1601 ppm to 3000 ppm

- HZn significantly improved the *ZO-1* and reduced the *IFN-γ* values.
- Pro-inflammatory cytokines, including *IFN-γ* are usually up regulated in the gut of newly weaned pigs; most of them like IFN-γ and IL-1β, have been shown to cause a weakening of the intestinal tight junctions (TJ) and increase gut permeability (Al Sadi et al., 2009).

Al-Sadi et al., Front Biosci. 2009;14: 2765–2778

HZn confirms its effect in improving the intestinal morphology of small intestine.

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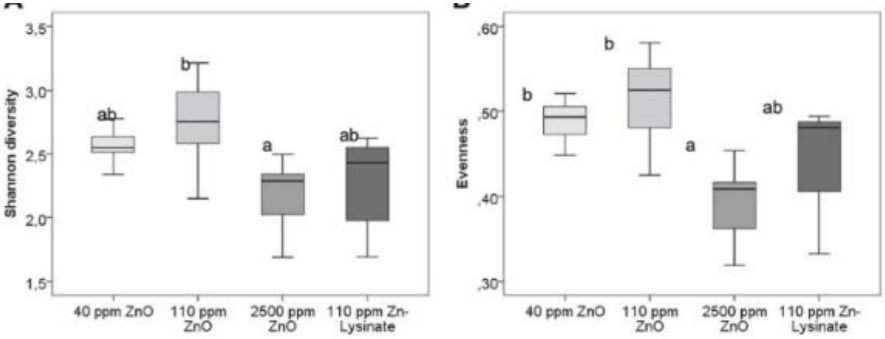
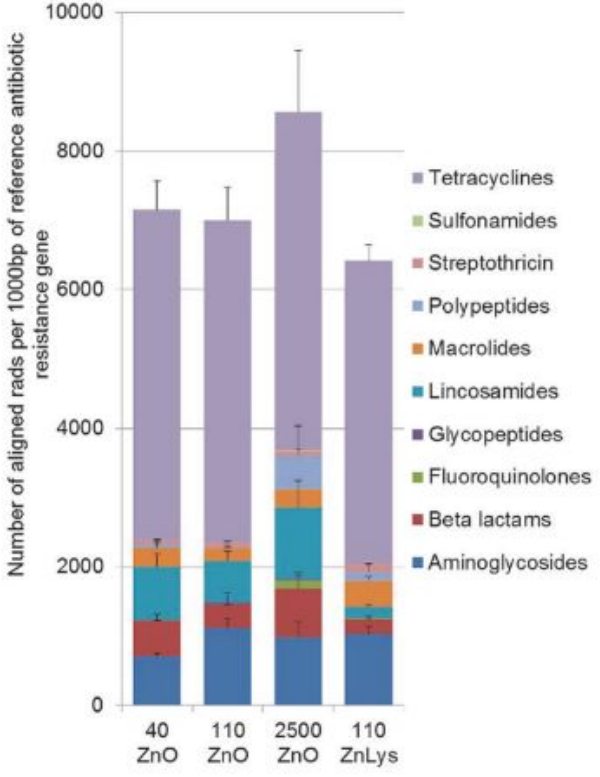
Effect of ZnO on gut microbiota

Effect of feed 3 weeks of zinc oxide at 40 ppm (40ZnO), 110 ppm (110ZnO), 2500 ppm (2500ZnO), or 110 ppm Zn-Lysinate (110ZnLys) on colon microbiome – METAGENOMIC



ZnO 2500 reduce d the microbial diversity and can increase the resistance of bacteria to specific antimicrobials



Effect of ZnO at different level on intestinal bacteria

ITEM	N. studies	LZn	M1Zn	M2Zn	HZn	SEM	P-value		
							Zn level classes	Basal Zn	Ref
E. coli, feces	4	100.25	96.68	/	97.01	1.39	0.18	0.20	-
Lactobacillus, feces	4	95.57	103.71	/	94.81	22.76	0.91	0.74	0.23
E. coli, large intestine	5	100.05	103.20		92.42	4.11	0.12	0.89	0.25
Lactobacillus, large intestine	4	100.01	99.78		99.85	2.25	0.98	0.38	0.46
E. coli, small intestine	6	73.72^{b}	/	/	231.88^{a}	62.39	0.04	0.55	0.20
Lactobacillus, small intestine	6	104.04^{a}	98.74^{ab}		81.23^{b}	10.31	0.05	0.63	0.18

LZn: 0 ppm to 200 ppm; M1Zn:201 ppm to 630 ppm; M2Zn: 631ppm to 1600 ppm; HZn from 1601 ppm to 3000 ppm

ZnO has a low solubility in the large intestine due to high pH, which generally leads to no effect on colonic microbiota (*Pieper et al.*, 2012; *Starke et al.*, 2014)

Zn can interact with specific components of the cell membrane structure of the Gram+; therefore it is more effective in reducing the Gram+ than the Gram- abundance (*Tayel*, 2011).

Tayel at al., 2011; J. Food Safety, 31, 211-218

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Gut health concept

Oxidative stress occurs when the production of reactive oxygen species (ROS) such as superoxide is not balanced by antioxidant defence.

- Total glutathione and Oxidized glutathione concentration
- Antioxidative enzyme

The microbiota balance could be defined as the opposite of dysbiosis meaning "a situation in which the microbial population is abundant and diversified with a high abundance of beneficial microorganisms than pathogenic ones"

- Alpha and beta diversity
- An abundance of beneficial or harmful bacteria, viruses and parasites

Capacity to digest, absorb and be functional

- Gut morphometry
- Levels of tight junctions
- Cell proliferation/ cell apoptosis
- Digestive enzyme activity

Immune fitness could be defined as "the ability of the host immune system to respond appropriately to a challenge and to return to or remain in the homeostatic immune state in the absence of a challenge.

- Immunoglobulin concentrations
- Cytokines concentration
- Lymphocytes proliferation

Chalvon-Demersay. 2021. Front. Vet. Sci. 8:663727. doi: 10.3389/fvets.2021.663727

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Feed additives and some more…

FEED ADDITIVE

(Regulation 1831/2003/EC)

- ✔ Improve the quality of the feed and of the food of animal origin
- ✔ Positively affect the performance and welfare of the animals in good health.

FEED MATERIALS

(Regulation (UE) N. 68/2013)

- ✔ Include also products that not meet a claim of the regulation 1831/2003 but that can have a role on the animal physiology.

VETERINARY MEDICINES

(Regulation 2019/6, from 28.01.22)

- ✔ Treating or preventing disease
- ✔ Restoring, correcting, modifying physiological functions

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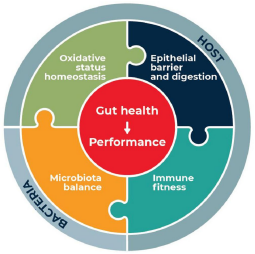
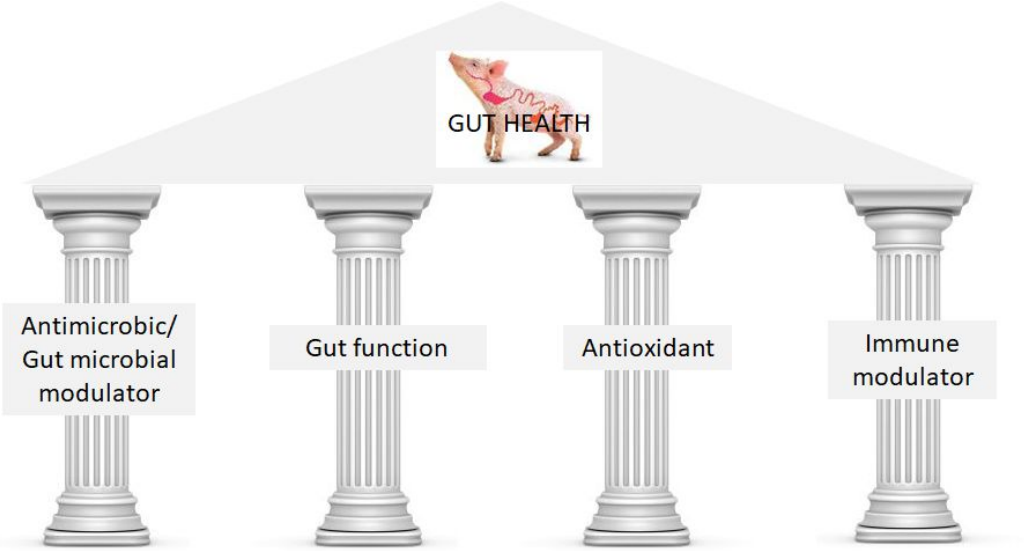
Feed additive and gut health





- Probiotic
- Polyphenols
- Organic acids
- Enzymes

- Polyphenols
- Organic acids
- Enzymes
- Amino acids

- Vitamins
- Essential oils
- Polyphenols
- Amino acids

- Essential oils
- Vitamins
- Probiotic
- Amino acids

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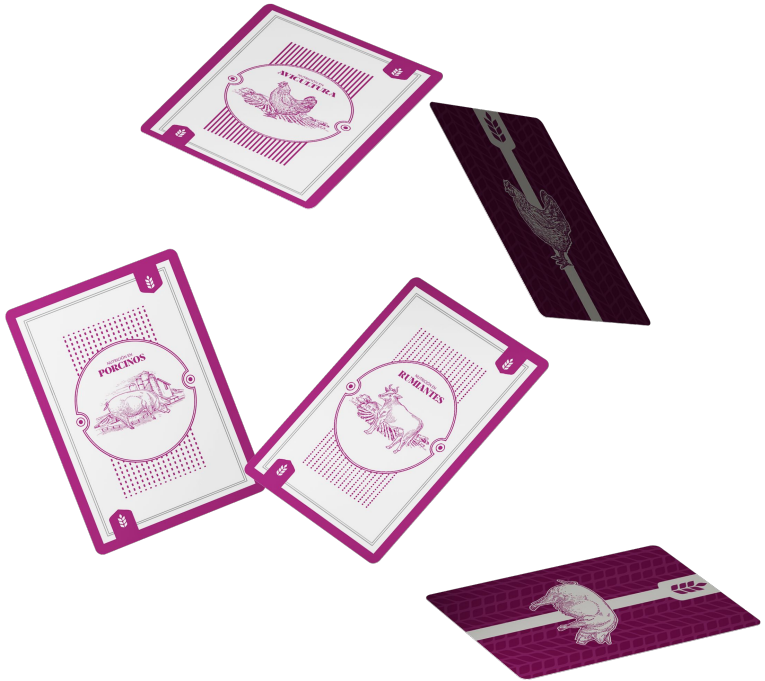
Organic acids, short and medium fatty acids

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



Organic acids

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	$CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$

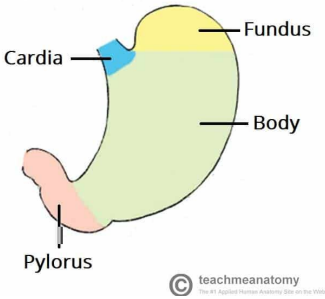
Short

Medium

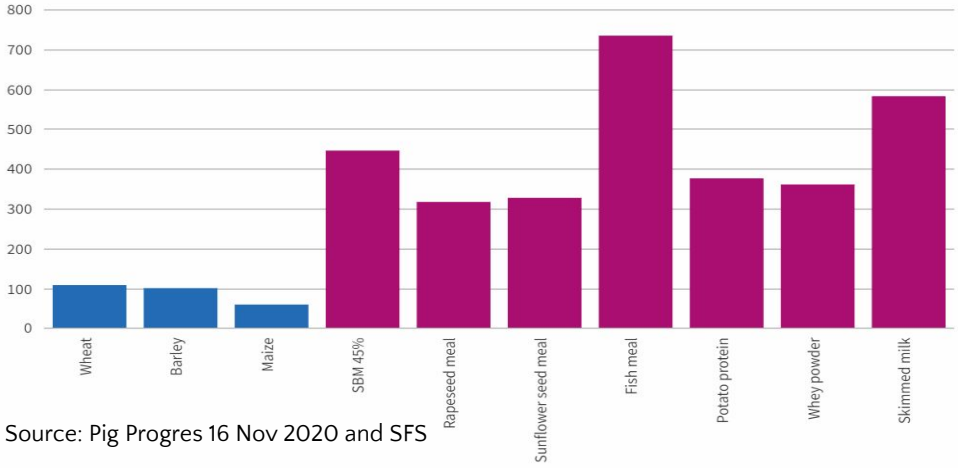
At weaning, physiological immaturity of the gastric mucosa (fundus) □ low HCl secretion by parietal cells

Interaction between gastric acid secretion capacity and diet:

- □ acid-binding capacity
- □ buffering capacity



Buffer capacity of different ingredients (mEq/kg)



Source: Pig Progres 16 Nov 2020 and SFS

Raised stomach pH

⇩

reduced digestion of feed

⇩

Increased risk of gut pathogen colonization

⇩

Risk of diarrhoea

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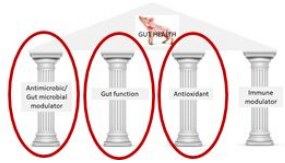
Organic acids: Formic acid and its salts



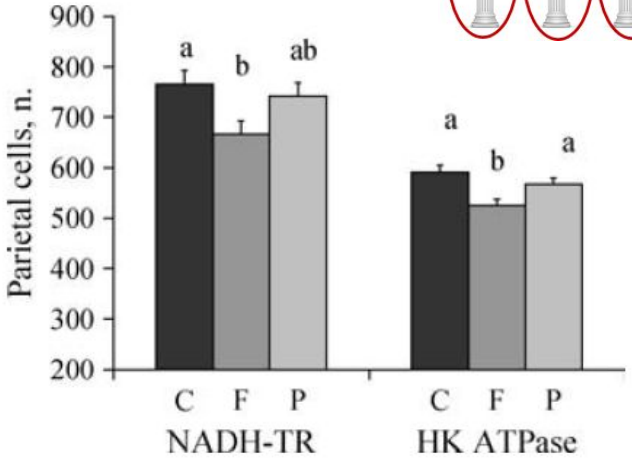
Considering formic acid, a dose of **8000–10000 mg/kg** of feed for improving the health and performance of weaning pigs (*Luise et al.* 2020).

The effect of formic acid and its salts on microbiome seems to be more consistent in the large intestine, while in the small intestine, results are varying accordingly to their doses and forms.

Acidifier [1]	Inclusion g/kg Diet	Initial Body Weight (BW)	Changes in Microbial Counts, log10 Colony-Forming Units (CFU)												References
			Stomach			Small Intestine			Large Intestine			Feces			
			Total	LAC	COLI	Total	LAC	COLI	Total	LAC	COLI	Total	LAC	COLI	
Formic acid	6	6					↑	↑		↓	↓				[66]
	12	6					↓	↓		↓	↓				[66]
	18	6					↓	↑		↓	↓				[66]
	24	6					↓	↓		↓	↓				[66]
	7						↓	↓							[53]
	14			-	↓		↓	↓							[53]
Ca-formate	18	7.5					↑	↑		↑	↓				[67]
Na-diformate	18	5.7					↓	↓		↓	↓				[68]
K-diformiate	18		↓	↓	-	↓	↓	-	↓	↓	-				[69]
	18	8			↓		-	↓							[70]
	5	7.8											↑	↓	[71]
Formic + Lactic acid	1 0+ 10	8.4	↓	↓	↓										[72]
Blend_1	4	6.7				↓	↓		-	↓					[73]
Blend_2	11	4.9					-	↑		↓	↑				[74]
Blend_3	21	4.9					-	↑		↓	↑				[74]
Blend_4	3												-	↓	[7]

[1] Blend_1: 35% formic acid + 35% lactic acid + 20% citric acid + 10% sorbic acid; Blend_2: 23.1% formic acid + 13.3% lactic acid + 12.4% acetic acid + 0.76 phosphoric acid + 0.76 citric acid; Blend_3: 51.7% lactic acid + 29.0% formic acid + 17.0% acetic acid + 16.0% phosphoric acid + 0.85% citric acid; Blend_4: commercial blend of free and buffered short chain fatty acids (mainly formic acid, acetic acid and propionic acid) combined with MCFA.

Luise et al., Animals. 2020;10.



C: control group, fed a standard weaning pigs; F: C+ 1.2% of free calcium formate; P: C+ 1.2% of fat-protected calcium formate

The reduction in the number of parietal cells could impair the absorption of vitamin B-12 due to a reduced secretion of the intrinsic factor by these cells

Bosi et al. 2006. J. Nutr. 136: 1229–1235

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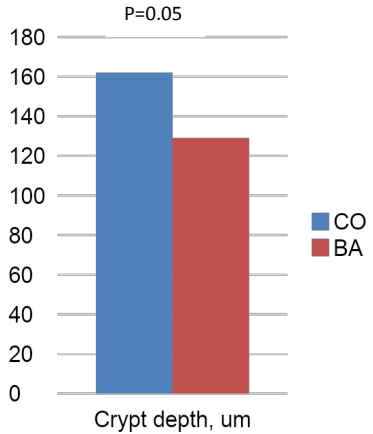
Organic acids: Benzoic acid

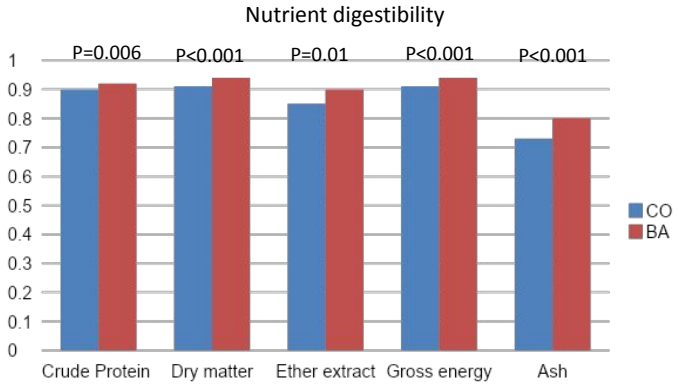
Effect of 5000 mg/kg benzoic acid on nursery pigs (18 kg) after 14 days of supplementation

Effect of benzoic acid on pH and enzymatic activity in jejunum mucosa

Items	Control	Benzoic acid	SEM	*P*-value
pH	6.2	5.7	0.11	0.064
Trypsin,U•10^3/mg protein	45.3^b	48.2^a	0.64	0.039
Lipase,U•10^3/g protein	2.7^b	2.9^a	0.32	0.028
Amylase,U/mg protein	309.1^b	401.9^a	15.81	0.030



Diao et al. Journal of Animal Science and Biotechnology (2016) 7:32



Effect of benzoic acid on gut oxidation in jejunum

Items	Control	Benzoic acid	SEM	*P*-value
Superoxide Dismutase,U/mL	29.07^b	70.43^a	5.73	0.011
Glutathione peroxidase,U/mg	83.08^b	345.97^a	22.88	0.049
Malondialdehyde,μmol/L	1.71	1.52	0.09	0.322

5000 mg/kg benzoic acid

- improve the nutrient digestion
- Improve gut morphology
- Increasing the antioxidant capacity

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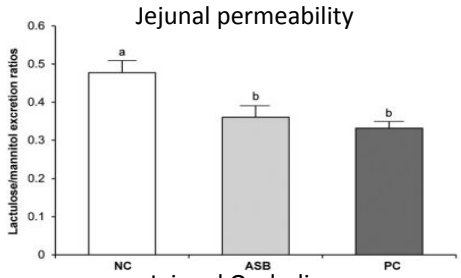
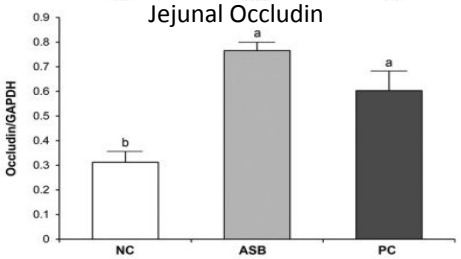
Organic acids: Coated sodium-butyrate

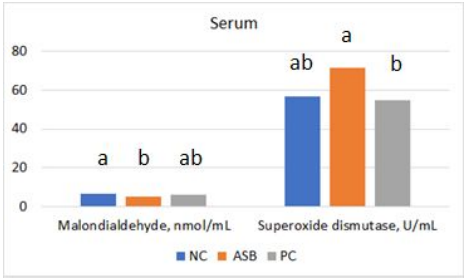
Coated Sodium Butyrate on jejunum morphology 42 days post-weaning

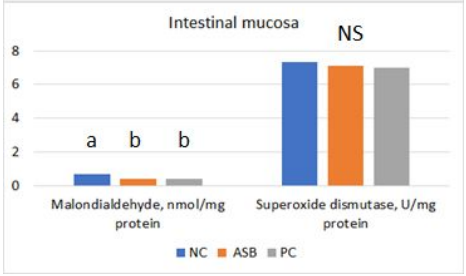
Items, μM	CON	Coated sodium butyrate			SEM[b]	P-value	
		Low	Medium	High		Linear	Quadratic
Duodenum	340.8	341.9	348.3	364.0	6.14	0.021	0.266
Jejunum	306.8	353.6	363.0	354.1	11.99	0.019	0.045
Ileum	303.7	360.3	363.0	352.3	11.28	0.016	0.015

Low: 0.5 g/ton SB; **Medium**: 1.5 g/ton SB and after 3 weeks 0,75 g/ton SB; **High**: 3 g/ton SB and after 3 weeks 1,5 g/ton SB









NC= Control; ASB= NC + 50 mg kitasamycin/kg, 20 mg colistin sulfate/kg, and 1000 mg encapsulated SB/kg; PC= NC+ 100 mg kitasamycin/kg and 40 mg colistin sulfate/kg [positive control]

Coated sodium butyrate

- improve gut morphology
- Improve gut barrier function and reduce the gut permeability
- Increasing the antioxidant capacity

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Huang et al., 2015; J.Nutri, 145, 2774-80 *Upadhaya et al.*, 2020; Anim Feed Sci Technol. 265,114534

Blends of short and medium chain fatty acids

SCFA and MCFA are usually mixed in blends together and/or with other additives to improve their activities following the approach called «multi-hurdled» proposed in humans (Rostami et al., 2016)

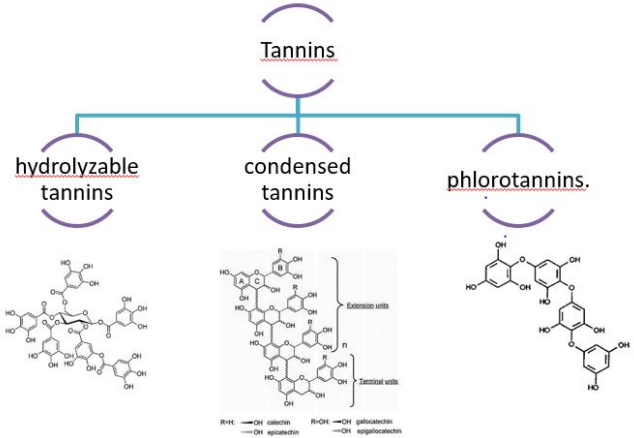
Item	CTR1	AGP	OA1	OA2	P value	REF
ADG, g/d	295ab	281b	314ab	332a	**0.03**	*Long et al.*, 2018
FE	502	478	476	488	0.48	
Diarrhoea rate	3.58a	1.59b	0.60b	0.40b	**<0.01**	
Blood, IgM, g/l	0.57b	0.65ab	0.97a	0.70ab	**0.05**	
Escherichia coli, feaces	4.50a	2.60b	3.36b	2.87b	**0.04**	
Total volatile fatty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

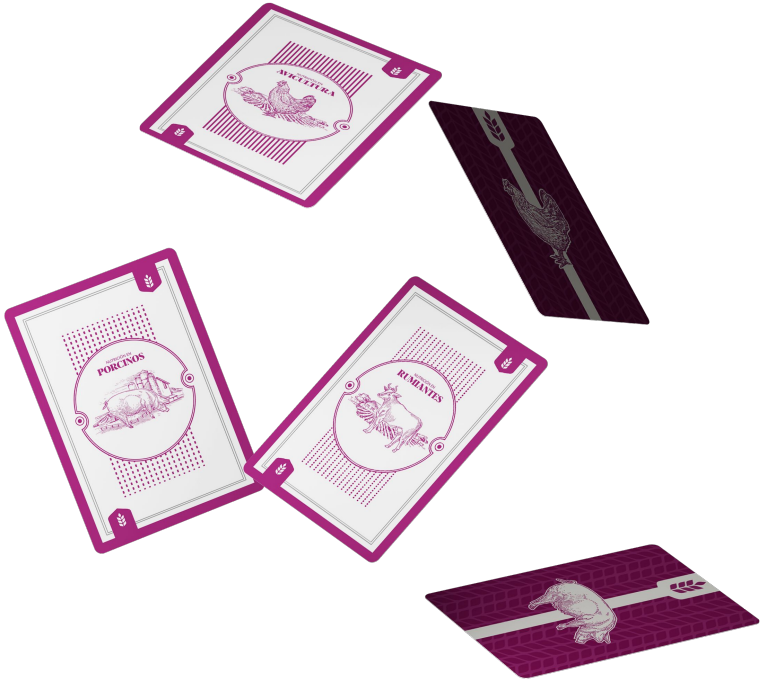
	NC	PC	AGPs	OAMF1	OAMF2	P value	REF
ADG	163 a	164 a	181 b	182 b	177 ab	**0.034**	*Han et al.*, 2020
FE	45	356	378	375	360	0.594	
Faecal score	1.96 a	1.93 a	1.46 d	1.60 c	1.71 b	**< 0.001**	
IgM (g/L)	2.25 a	2.35 ab	2.37 bc	2.42 c	2.32 ab	**0.009**	
IgG (g/L)	18.91 a	18.56 a	19.44 a	21.04 b	21.44 b	**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

Nutri Forum 23

Tannins

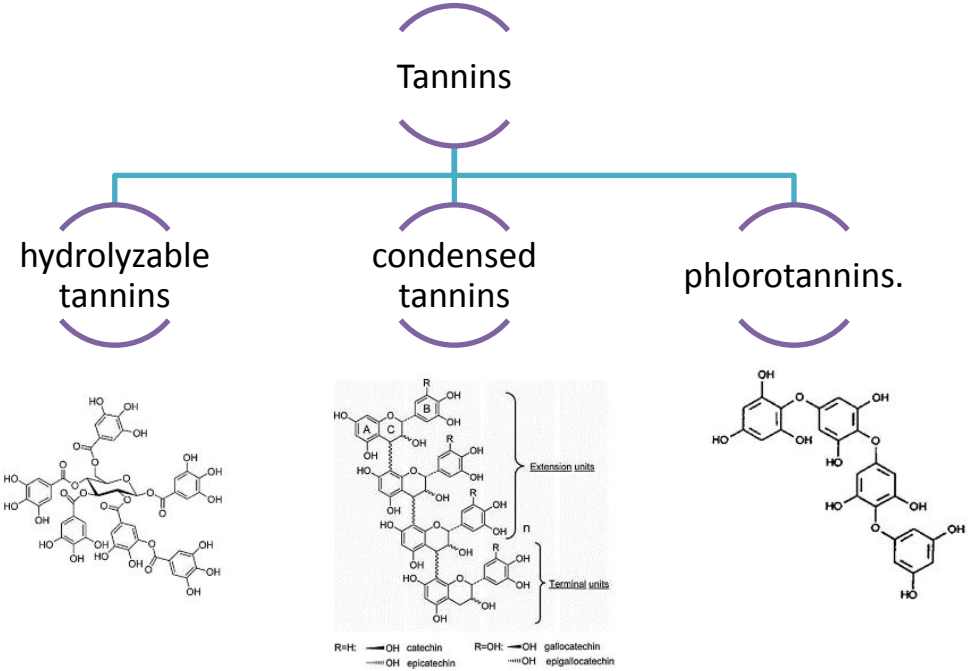

Tannins
hydrolyzable tannins
condensed tannins
phlorotannins.


AVICULTURA
PORCINOS
RUMIANTES

Tannins

Tannins are a heterogeneous group of astringent polyphenolic biomolecules that can interact with and precipitate macromolecules, such as proteins, gelatins, polysaccharides and alkaloids.

Tannins are widely distributed in plant:
-forages, shrubs, cereals and medicinal herbs
- fruit species such as banana, blackberry, apple and grape as well as tea



Girard and Bee. Animal. 2020;14:95–107.

Nutri Forum 23

Tannins antimicrobial effects - *in vitro*

Plant source	Plant part	Tannins (CTs or HTs)	Tannin or metabolite type	*In vitro* and/ or *in vivo*	Bacteria	Reference
Pomegranate (*Punica granatum L.*)	Fruit peel	HTs	Ellagitannins (punicalagin) Ellagic acid (metabolite)	*In vitro*	Food-borne pathogens: *Escherichia coli, Listeria monocytogenes, Staphylococcus aureus, Yersinia enterocolitica*	Al-Zoreky (2009)
Chestnut (*Castanea sativa*)	Wood	HTs	Gallotannins Ellagitannins	*In vitro*	Poultry pathogens: *Campylobacter jejuni, Clostridium perfringens* type A, *Escherichia coli, Pasteurella multocida, Salmonella enteritidis, Salmonella gallinarum, Salmonella typhimurium, Salmonella virchow, Staphylococcus aureus*	Graziani *et al.* (2006)
Japanese rose (*Rosa rugosa*)	Petals	HTs	Ellagitannins (tellimagrandin II, rugosin A and D)	*In vitro*	Intestinal bacteria: *Bacillus cereus, Escherichia coli, Salmonella* sp., *Staphylococcus aureus*	Kamijo *et al.* (2008)
Chestnut (*Castanea sativa*)	Not specified	HTs	Gallotannins Ellagitannins	*In vitro/ in vivo*	*Escherichia coli* O157 : H7 (*in vitro*) and generic fecal *Escherichia coli* (*in vivo*)	Min *et al.* (2007)
Mimosa (*Acacia mearnsii*)	Not specified	CTs	Procyanidins Prodelphinidins Prorobinetinidins			
Sumac (*Rhus copallina*)	Leaves	CTs : HTs (17% : 83%)	Gallotannins Ellagitannins	*In vitro*	*Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus*	Min *et al.* (2008)
Shinnery oak (*Quercus havardii*)	Leaves	CTs : HTs (29% : 71%)	Catechin (flavan-3-ol) Gallotannins Ellagitannins			
Quebracho extract (*Schinopsis* spp.)	Not specified	CTs : HTs (98.5% : 1.5%)	Profisetinidins Prorobinetinidins			
Japanese chestnut (*Castanea crenata*)	Wood	HTs	Castalagin	*In vitro*	*Escherichia coli* (non-pathogenic *E. coli*, enterohemorrhagic *E. coli*, enteroinvasive *E. coli*, enterotoxigenic *E.coli*), *Salmonella, Staphylococcus aureus*	Taguri *et al.* (2004)
Woodland elaeocarpus (*Elaeocarpus*	Bark	CTs	Prodelphinidins			

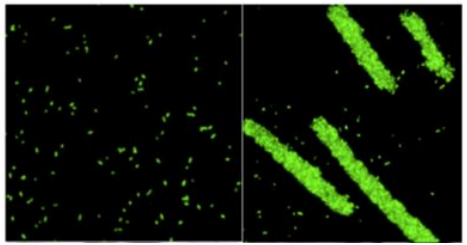
inhibition of extra-cellular microbial enzymes

deprivation of the substrates required for microbial growth

inhibition of oxidative phosphorylation

deprivation of metal ions

formation of complex with the cell membrane

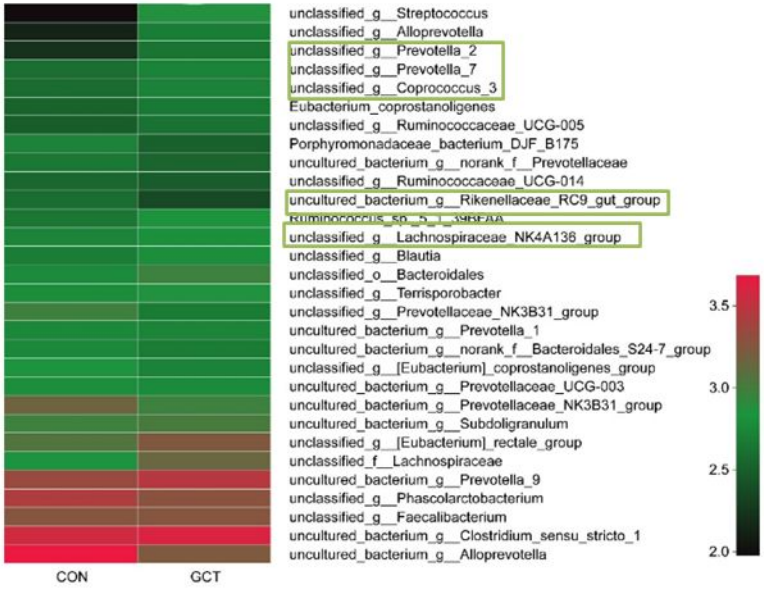


Aggregation of *Escherichia coli* (strain 25922) cell incubated for 10 h with Control or 200 mg/mL of condensed tannins of purple prairie clover (Dalea purpurea Vent.). *Huang et al.*, 2018, Animal Nutrition, 4, 137-150.

Nutri Forum 23

Tannins: *in vivo* effects

Effect of 21 days feeding with 1.8% of tannin extract (from Galla chinensis :effective content of tannic acid was 70.38%) on the cecal microbiota, gut morphology and blood oxidative stress parameters – comparison with 1500 ppm of ZnO



Sun et al. Animals. 2021;11.

Item	CON	GCT	SEM	*p* Value
Villus height (um)				
Duodenum	454.25	401.02	15.63	0.088
Jejunum	422.44	410.70	16.99	0.747
Ileum	384.67	399.66	14.95	0.639
Crypt depth (um)				
Duodenum	142.70	148.20	2.90	0.367
Jejunum	159.04	150.06	4.02	0.284
Ileum	144.69	128.25	4.08	0.036
D-lac	1288	1106	36.29	0.004

CON: ZnO diet; GCT: Hydrolyzed Chinese gallnut tannic acid diet; D-lac: D-lactic acid; SEM: Standard error of the mean.

Item	CON	GCT	SEM	*p* Value
GSH (mg/mL)	5.57	6.43	0.19	0.012
MDA (nmol/mL)	4.15	3.58	0.14	0.032
SOD (U/mL)	97.16	105.47	2.06	0.036

CON: ZnO diet; GCT: Hydrolyzed Chinese gallnut tannic acid diet; GSH: Glutathione; MDA: Malondialdehyde; SOD: Superoxide dismutase.

Tannin extract:

- ✔ Favour the colonisation of the gut intestine with beneficial bacteria
- ✔ improve the barrier integrity and reduce the oxidative stress in piglets

Nutri Forum 23

Tannins: *In vivo* effects

Effect of feeding from 28 days 1% of tannin extract from chestnut wood and contained ≥75% tannin, crude fiber <8.00%

Table 5. Effects of HT and ZnO individual supplementation or combination on serum antioxidant capacity of weaned piglets.

Item [1]	CON [2]	ZnO [2]	HT [2]	HT + ZnO [2]	SEM [3]	*p*-Value
			Duodenum			
T-AOC (U/mg)	6.90 ^{b}	8.72 ^{ab}	8.90 ^{ab}	10.70 ^{a}	0.55	<0.01
CAT (U/mg)	3.67	4.17	5.02	5.51	0.42	0.05
GSH-Px (U/mg)	78.98 ^{b}	83.04 ^{b}	89.06 ^{ab}	116.72 ^{a}	6.57	0.01
SOD (U/mg)	6.41 ^{b}	6.39 ^{b}	7.80 ^{a}	8.48 ^{a}	0.29	<0.01
MDA (nmol/mg)	0.61 ^{a}	0.57 ^{ab}	0.55 ^{ab}	0.46 ^{b}	0.03	0.05
			Jejunum			
T-AOC (U/mg)	8.62	8.12	7.11	7.20	0.23	0.22
CAT (U/mg)	2.66	2.35	2.87	2.90	3.53	0.37
GSH-Px (U/mg)	61.56	47.58	51.38	63.64	0.21	0.07
SOD (U/mg)	6.12	5.70	6.16	6.42	0.55	0.17
MDA (nmol/mg)	0.66	0.79	0.74	0.70	0.03	0.07
			Ileum			
T-AOC (U/mg)	8.18	8.54	8.51	6.54	0.81	0.29
CAT (U/mg)	4.16	4.49	4.49	4.28	0.42	0.82
GSH-Px (U/mg)	77.71 ^{ab}	69.71 ^{b}	94.13 ^{a}	79.30 ^{ab}	4.66	0.03
SOD (U/mg)	7.37	6.80	7.57	6.87	0.38	0.43
MDA (nmol/mg)	0.58 ^{ab}	0.63 ^{a}	0.53 ^{b}	0.59 ^{ab}	0.02	0.03
			Liver			
CAT (U/mg)	5.21 ^{b}	7.17 ^{a}	6.04 ^{ab}	5.49 ^{b}	0.27	<0.01
GSH-Px (U/mg)	71.56	87.26	86.16	77.79	6.19	0.32
SOD (U/mg)	7.06 ^{b}	9.09 ^{a}	8.60 ^{ab}	8.01 ^{ab}	0.36	0.03
MDA (nmol/mg)	0.62	0.56	0.57	0.59	0.02	0.36

CON= control; ZnO= CON + 2000 mg/kg ZnO in phase 1 and 137.5 mg/kg ZnO in phase 2; HT= CON + 1000 mg/kg HT in all phase; HT+ ZnO=CON + 2000 mg/kg ZnO + 1000 mg/kg HT in phase 1, and 137.5 mg/kg ZnO + 1000 mg/kg HT in phase 2 (HT + ZnO.

Liu et al., 2020.

Table 4. Effect of HT and ZnO individual supplementation or combination on serum antioxidant and immune indexes of weaned pigs.

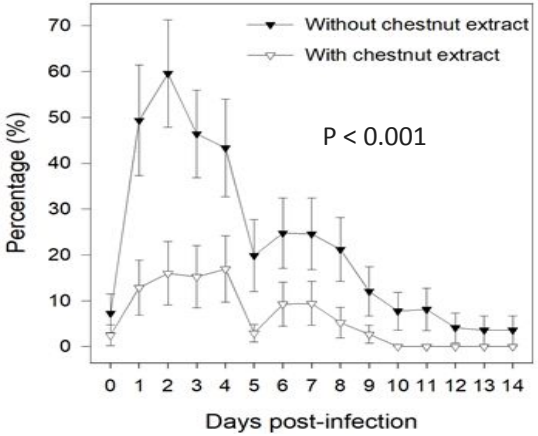
Item [1]	CON [2]	ZnO [2]	HT [2]	HT + ZnO [2]	SEM [3]	*p*-Value
			d 14			
CAT(U/mL)	24.36 ^{c}	29.18 ^{bc}	33.19 ^{b}	47.96 ^{a}	1.17	<0.01
GSH-Px (U/mL)	684 ^{c}	855 ^{b}	919 ^{b}	1049 ^{a}	24.80	<0.01
SOD (U/mL)	71.72 ^{ab}	66.14 ^{ab}	64.16 ^{b}	73.95 ^{a}	2.21	0.03
T-AOC (U/mL)	13.31 ^{ab}	12.80 ^{ab}	12.78 ^{b}	15.90 ^{a}	0.97	0.02
MDA (nmol/mL)	5.48 ^{a}	4.84 ^{ab}	4.63 ^{b}	2.71 ^{c}	0.20	<0.01
IgA (g/L)	1.16 ^{b}	1.38 ^{ab}	1.42 ^{ab}	1.60 ^{a}	0.09	0.03
IgM (g/L)	3.13 ^{c}	3.38 ^{bc}	3.48 ^{b}	3.96 ^{a}	0.06	<0.01
IgG (g/L)	20.67	21.25	21.56	23.11	0.62	0.08

- ✔ HT may alleviate the oxidative stress by enhancing the antioxidant enzyme activities in serum and small intestine, which was reflected by improved **GSH-Px** activities and reduce **MDA** in the serum and ileum.
- ✔ The possible reason is that HT can selectively induce the expression of antioxidant enzymes via **modulating redox-sensitive signalling pathways** by inhibiting lipid peroxidation and quenching the oxygen free radicals in the gut.
- ✔ Seems there is a synergistic effect between ZnO and HT

Nutri Forum 23

Tannins: *In vivo* effects

Girard et al., 2020 *Plos one*, *15*(2), e0214267



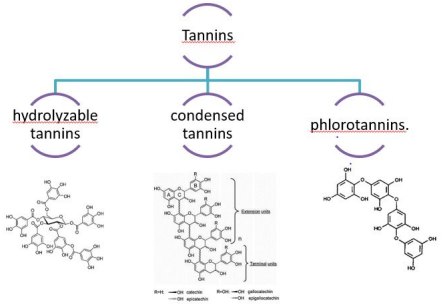
1% of HTs/CT reduced diarrhea incidence post-challenged with ETEC.

Source/type /feeding	Animals/ duration (day)	Dose of tannin	Zootechnical performances	References
Chestnut/HTs/ad libitum	12/28	Control, 0.11% HTs, 0.23% HTs, 0.45% HTs	= BW, ADG, ADFI, FCR with 0.11%, 0.23%; = BW, ADFI with 0.45%; ↑ ADG and FCR with 0.45%	Biagi et al. (2010)
Chestnut/HTs/ad libitum	168/104	Control, 0.35% 5 acids, 0.19% HTs + 0.16% 5 acids	↑ BW, ADG at 82 and 127 days; ↑ FCR at 82–127 days	Brus et al. (2013b)
Chestnut/HTs/restricted	8/14	Control, 0.075% HTs, 0.15% HTs, 0.3% HTs, Vit. E	= BW, ADG, ADFI, FCR	Frankič and Salobir (2011)
Chestnut and Quebracho/HTs/CTs/ad libitum	18/14	Control infected; control not infected; 1% HTs/CTs infected; 1% HTs/CTs not infected	= BW, ADG, ADFI, FCR	Girard et al. (2018)
Chestnut and Quebracho/HTs/CTs/ad libitum	36/14	Control without SA; 2% HTs/CTs; control with SA; SA + 2% HTs/CTs	↑ BW at 7 days; ↑ ADG, ADFI	Girard and Bee (2020)

- ✔ Doses of 2% HTs/CTs may result in improved daily feed intake and average body gain
- ✔ Doses under 1% HTs/CTs seem not to affect animals' growth performance
- ✔ Synergistic effect of lower dosage with organic acids have been observed by Brus et al. 2013

Nutri Forum 23

Tannins



However, tannins' beneficial effects depend primarily on their type and dose in the media or the diet.

Indeed, an excess of tannins in the diet could lead to an antinutritional effect, especially on proteins.

Further investigations are needed to determine the optimal combinations of tannins and the most suitable and cost-effective manner to deliver them.

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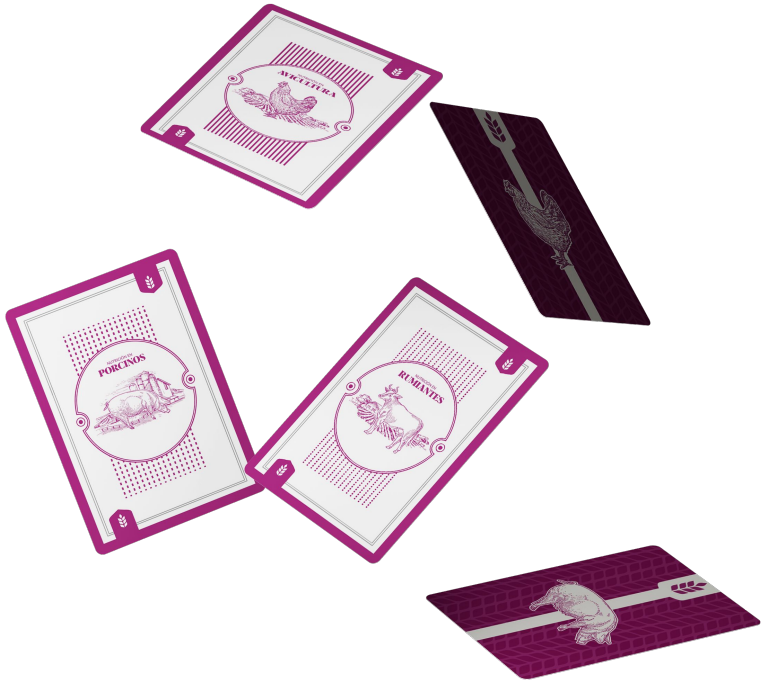
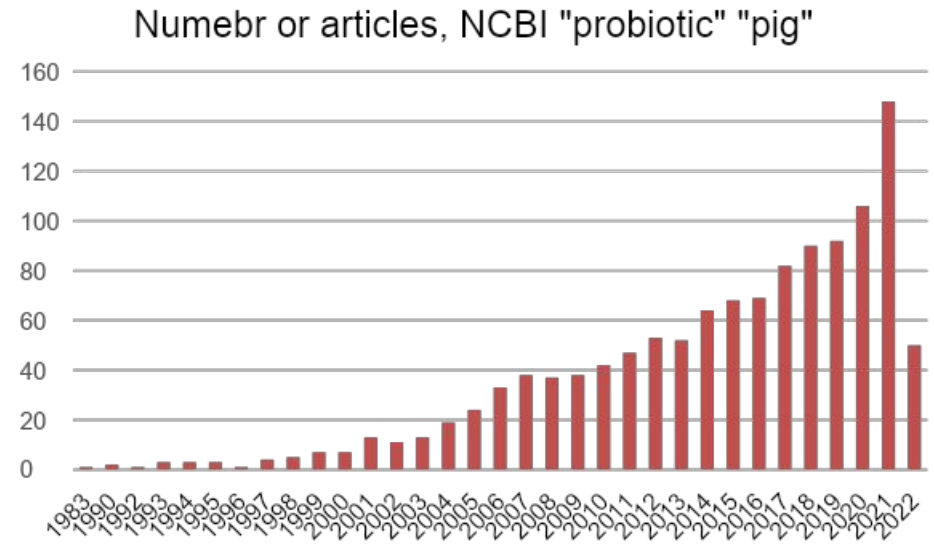
Probiotics

Genus	Strain
Pediococcus	*Pediococcus acidilactic*
Enterococcus	*Enterococcus faecium*
Saccharomyces	*Saccharomyces cerevisiae*
Bacillus	*Bacillus amyloliquefaciens*
	Bacillus licheniformis
	Bacillus subtilis
	Bacillus velezensis
	Bacillus coagulans
Clostridium	*Clostridium butyricum*

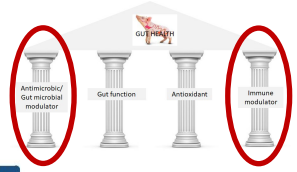


Probiotics



Hou et al. Journal of Animal Science and Biotechnology (2015) 6:14
DOI 10.1186/s40104-015-0014-3

JOURNAL OF ANIMAL SCIENCE AND BIOTECHNOLOGY



REVIEW Open Access

Study and use of the probiotic *Lactobacillus reuteri* in pigs: a review

Chengli Hou, Xiangfang Zeng, Fengjuan Yang, Hong Liu and Shiyan Qiao*

Livestock Science 223 (2019) 84–96
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REVIEW

Review article

Practical aspects of the use of probiotics in pig production: A review

Emili Barba-Vidal[a,b,*], Susana M. Martín-Orúe[a], Lorena Castillejos[b]

Bacillus spp. Probiotic Strains as a Potential Tool for Limiting the Use of Antibiotics, and Improving the Growth and Health of Pigs and Chickens

Pathogens **2015**, *4*, 34-45; doi:10.3390/pathogens4010034

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Beneficial Microbes, 2019; 10(7): 773-799

Wageningen Academic Publishers

Review

The Use of Lactic Acid Bacteria as a Probiotic in Swine Diets

Fengjuan Yang, Chengli Hou, Xiangfang Zeng and Shiyan Qiao *

Could probiotics be the panacea alternative to the use of antimicrobials in livestock diets?

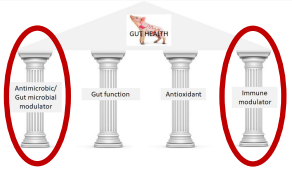
A. Cameron[1,2] and T.A. McAllister[2]*

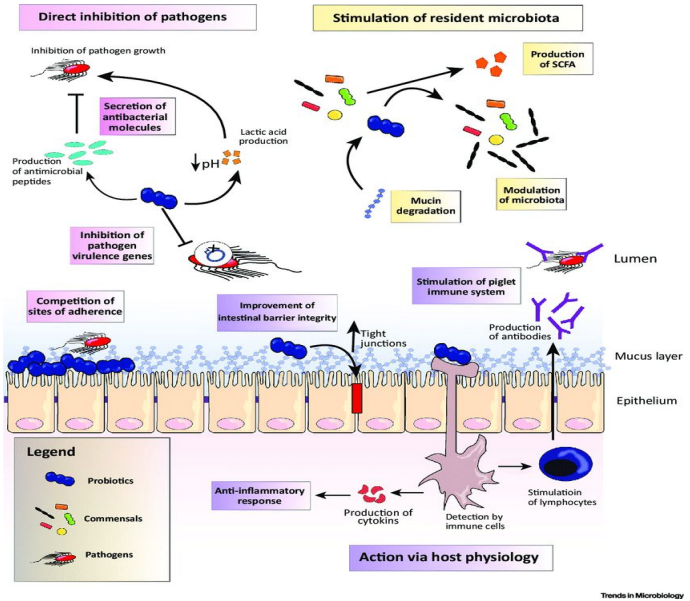
Nutri Forum 23

Probiotics

Genus	Strain
Pediococcus	*Pediococcus acidilactic*
Enterococcus	*Enterococcus faecium*
Saccharomyces	*Saccharomyces cerevisiae*
Bacillus	*Bacillus amyloliquefaciens*
	Bacillus licheniformis
	Bacillus subtilis
	Bacillus velezensis
	Bacillus coagulans
Clostridium	*Clostridium butyricum*

European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. Edition 03/2021 (291)

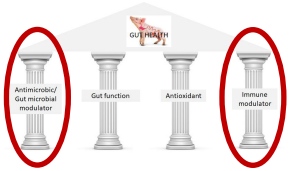




Gresse et al.,2017

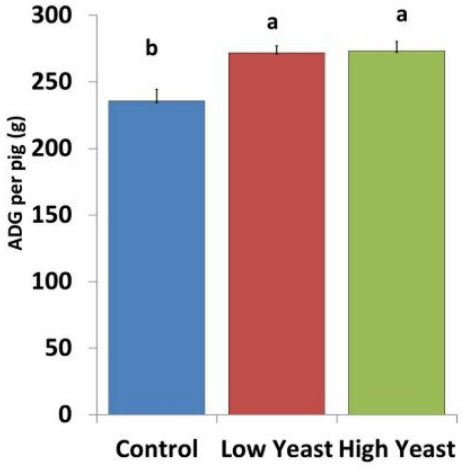
Nutri Forum 23

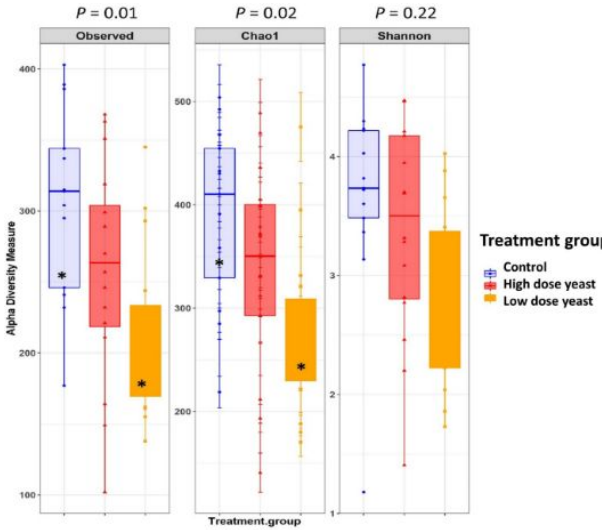
Probiotics – suckling phase, multiple doses

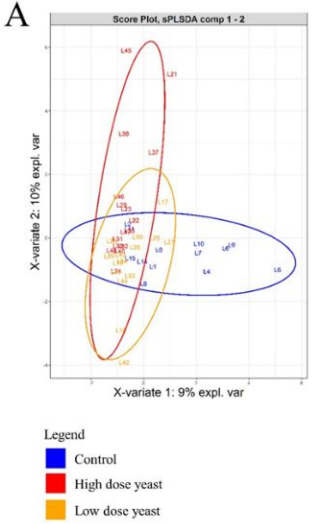


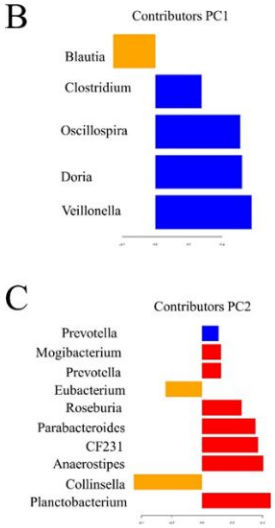
Directly supplemented to the neonate piglets : every other day from d1 of age until d28 (weaning), two doses *Saccharomyces cerevisiae*, 5 x 10^9 or 2.5 x 10^{10} CFU/piglet (*Kiros et al.*, 2019)

The supplementation of Saccharomyces cerevisiae ***increased the ADG*** *of piglets,* ***reduced the alpha diversity*** *indices and selected specific bacteria including SCFAs producing bacteria*



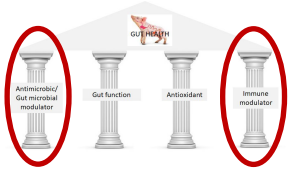






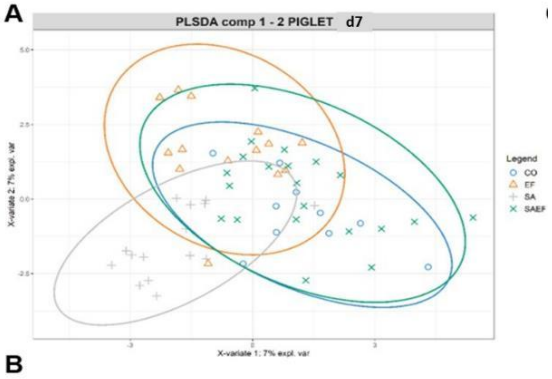
Kiros et al. 2019. PLoS ONE 14(7): e0219557.

Probiotics – suckling phase, single doses

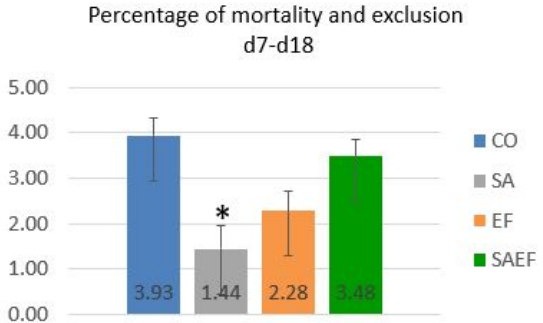


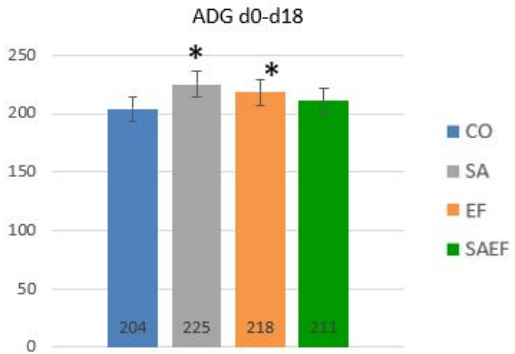
One shot on the day of birth only: orally inoculated 4mL containing: pure water (CO); 10^{10} CFU *Saccharomyces cerevisiae* boulardii (SA); 10^{10} CFU *Enterococcus faecium* lactiferm (SA); both (SAEF)

Effect on faecal microbiota



Value.var	Freq	PC	Genus	Direction
0.394	0.86	1	Prevotellaceae_UCG-004	CO
-0.360	0.93	1	Christensenella	SA
0.345	0.68	1	Romboutsia	SAEF
0.321	0.6	1	Ruminococcaceae_UCG-010	SAEF
-0.316	0.82	1	Catenibacterium	SA
0.249	0.72	1	Erysipelotrichaceae_UCG-004	SAEF
0.520	0.73	2	Lachnospiraceae_UCG-010	EF
-0.471	0.55	2	Erysipelatoclostridium	SA
0.289	0.46	2	Negativicoccus	EF
-0.282	0.51	2	Methanobrevibacter	SA
0.257	0.64	2	Lachnospiraceae_UCG-004	EF
-0.237	0.48	2	Lachnospiraceae_UCG-002	SA





These results highlighted that the administration of a single early-life probiotic supplement could improve piglet performance and shape the faecal microbial profile.

The two probiotic favoured different bacteria profile; suggesting that there was not a univocal stimulation of the microbiota for growth promotion.

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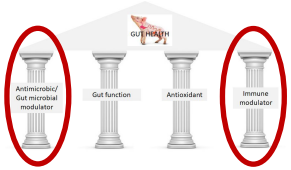
Luise et al., 2021; Italian Journal of Animal Science, 20:1, 1372-1385,

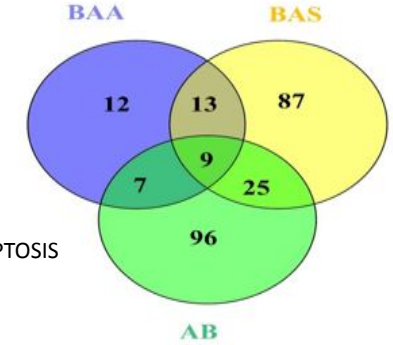
Probiotics – weaning phase



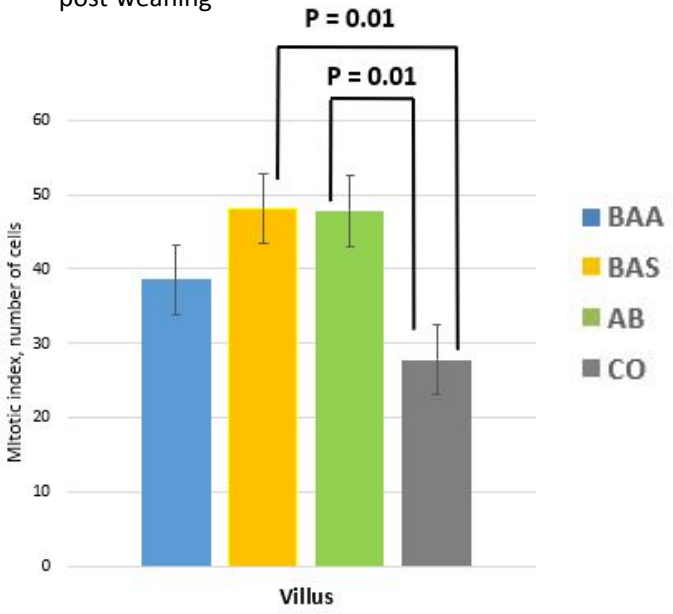
Enriched gene sets in Bacillus groups compared with a CO (FDR <0,25)

9 Gene sets of intersection AB|BAA|BAS

- LEUKOCYTE_ACTIVATION
- T_CELL_ACTIVATION
- LYMPHOCYTE_DIFFERENTIATION
- B_CELL_ACTIVATION
- T_CELL_DIFFERENTIATION
- CELL_STRUCTURE_DISASSEMBLY_DURING_APOPTOSIS
- DETECTION_OF_EXTERNAL_STIMULUS
- DETECTION_OF_STIMULUS



Mitotic index, d21 post-weaning



BAA: Bacillus Amyloliquefaciens; BAS: Bacillus subtilis; AB: colistin

Luise, et al., (2019). Journal of animal science and biotechnology, 10, 1-16.

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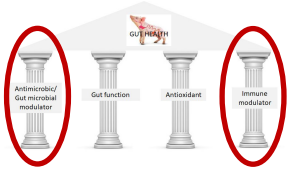
Probiotics – weaning phase



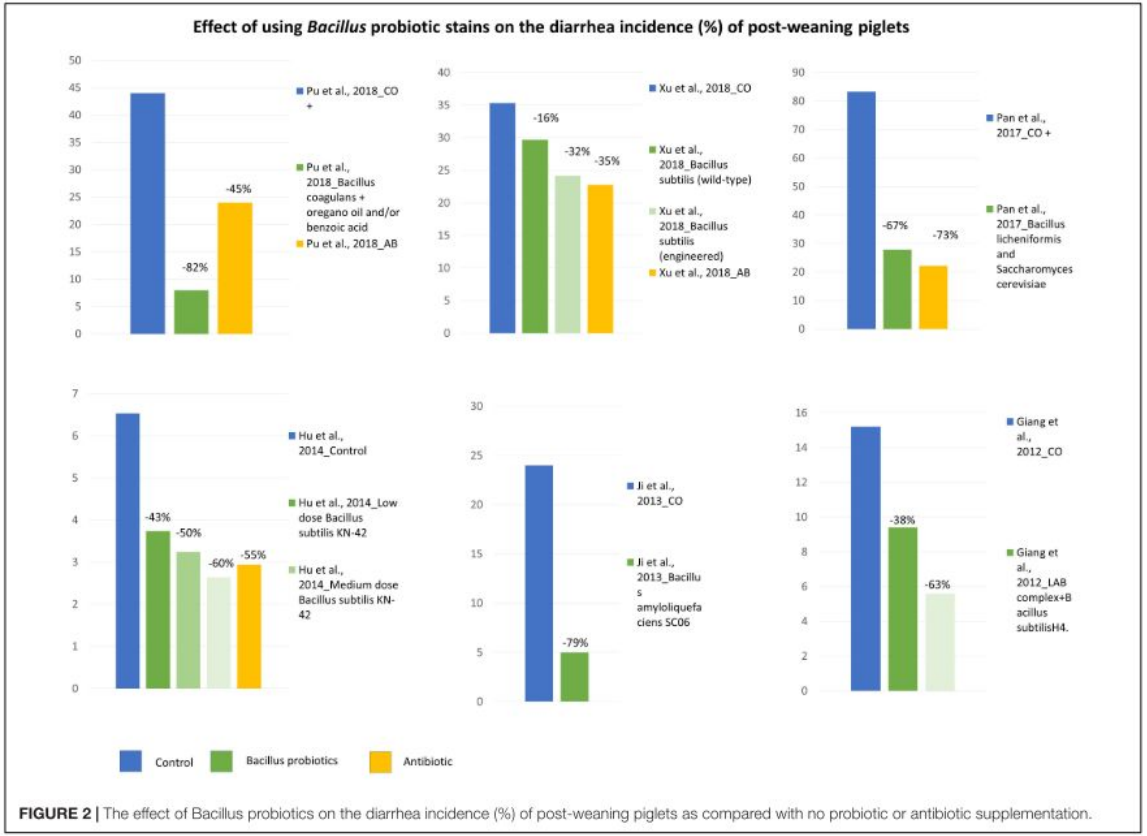


FIGURE 2 | The effect of Bacillus probiotics on the diarrhea incidence (%) of post-weaning piglets as compared with no probiotic or antibiotic supplementation.

Luise et al., (2022). Frontiers in Microbiology, 13, 177.

Bacillus strains reduced the **diarrhoea indices** (%) by at **least 30%** to values ranging from 79 to 82% (*Ji et al.,* 2013; *Pu et al.*, 2018) as compared with the untreated control groups.

Furthermore, the **reduction** in the **incidence of diarrhea**% achieved using Bacillus strains was **equal** (*Hu et al.*, 2014; *Pan et al.*, 2017; *Xu J. et al.*, 2017) **or greater** (*Pu et al.*, 2018) than the diarrhea% achieved using **antibiotics**.

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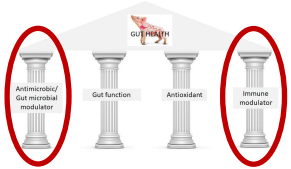
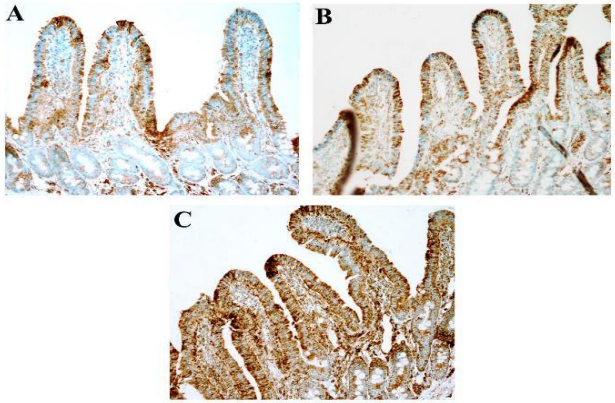
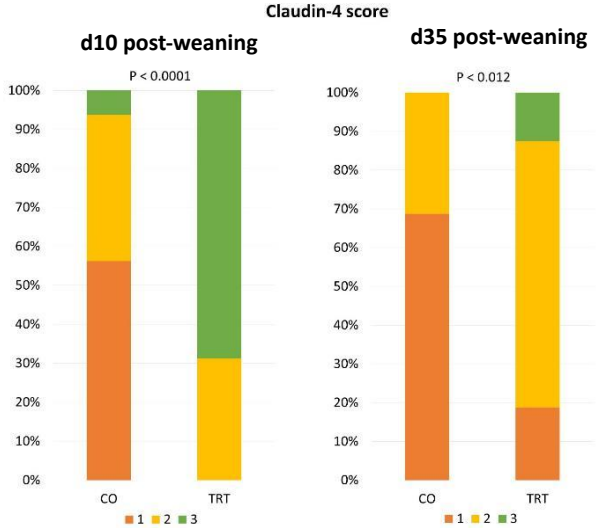
«Probiotics»



Effect of live oral vaccine against ETEC F4/F18 administered one time the day of the weaning on the gut health, in healthy piglets





Jejunal immunohistochemistry scoring for Claudin-4 of post-weaning piglets orally vaccinated with Coliprotec F4/F18. (*Correa et al.*, 2022, Animal, 100654)

Due to its role in the small intestine, claudin-4 can be considered a good marker for barrier function and mucosal integrity. The higher score observed in the vaccinated group suggested a positive influence of the vaccine on barrier function and mucosal integrity in the jejunum of the piglets. Indeed, Claudin-4 expression and score decrease with inflammation (*Ciro Galeano et al.*, 2015; *Curry et al.*, 2017).

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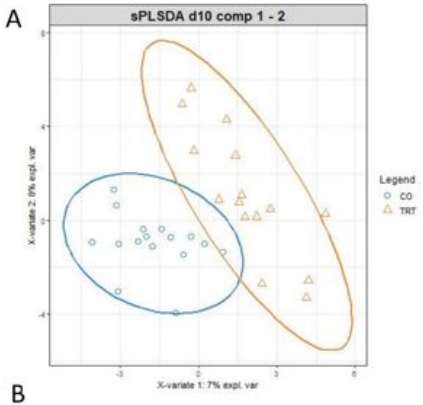
«Probiotics»

Effect of live oral vaccine against ETEC F4/F18 administered one time the day of the weaning on the gut health, in healthy piglets

The two vaccine strains showed a probiotic-like effect by modulating gut microbiota and favouring the establishment of beneficial bacteria, and by promoting gut barrier integrity.

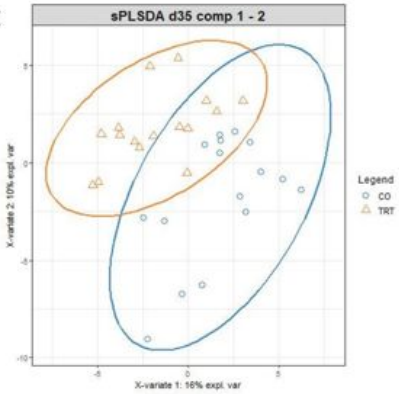
A



B

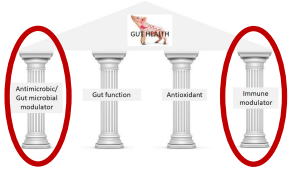
Group	value.var	Freq	PC	Taxa
CO	-0.29	0.60	2	Selenomonas
CO	-0.09	0.54	2	Mogibacterium
TRT	0.53	0.70	2	[Eubacterium] nodatum group
TRT	0.33	0.56	2	Prevotellaceae UCG-004
TRT	0.28	0.54	2	Escherichia-Shigella
TRT	0.91	0.77	1	Colidextribacter

C



D

Group	value.var	Freq	PC	Taxa
CO	0.13	0.54	1	Rikenellaceae RC9 gut group
CO	0.17	0.61	1	Lachnospiraceae XPB1014 group
CO	0.31	0.82	1	Monoglobus
CO	0.45	0.96	1	Family XIII UCG-001
CO	0.10	0.51	1	[Eubacterium] nodatum group
CO	0.28	0.81	1	Sutterella
TRT	-0.23	0.70	1	[Eubacterium] ruminantium group
TRT	-0.19	0.65	1	Acetitomaculum
TRT	-0.16	0.61	1	Alloprevotella
TRT	-0.13	0.57	1	[Bacteroides] pectinophilus group
TRT	-0.52	0.97	1	CAG-56
TRT	-0.16	0.61	1	Mitsuokella
TRT	-0.35	0.83	1	Subdoligranulum



(*Correa et al.*, 2022, Animal, 100654)

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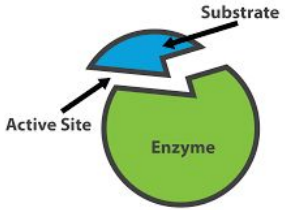
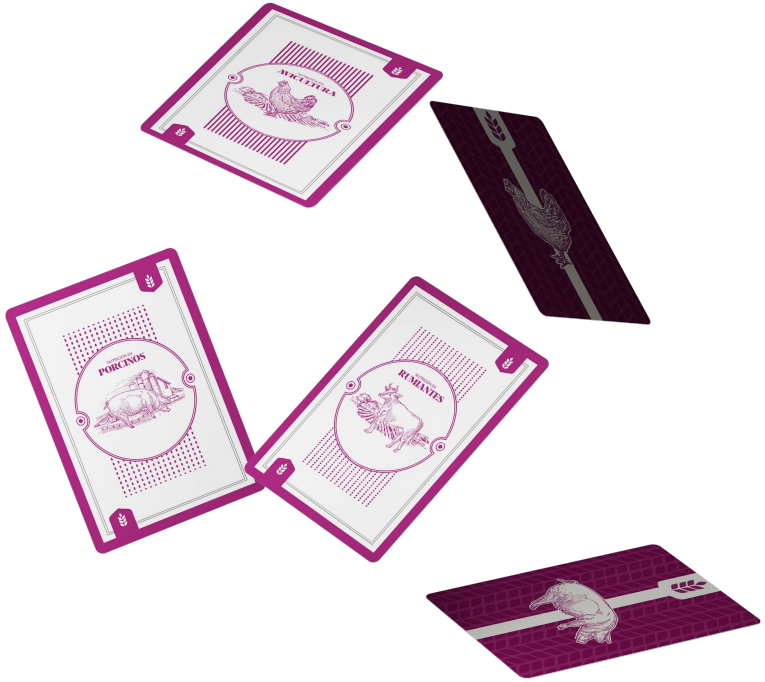
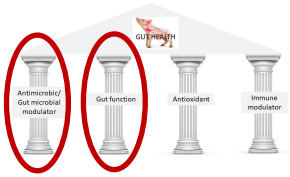
Enzymes and vitamins





Enzymes



- ✔ Enhance the endogenous enzymatic activity
- ✔ To compensate for any insufficiency and / or immaturity of the digestive system of young animals
- ✔ Degrading indigestible substrates of the vegetable raw material (phytates, non-starch polysaccharides, cellulose) for which animals does not secrete specific enzymes
- ✔ Reduce viscosity along the GIT
- ✔ Eubiotic effect

Enzymes tested	Doses in diets	Main substrateingredients	Duration (days)	Control	End-points[a b]							Reference
					GP	ND	FT/D	GA	IS	GM	AS	
Xylanase	2000 U/kg	Wheat			+	+						Walsh et al. (2014)
Amylase	100 – 350 mg EME/kg[1]	Corn-soybean	14 (digestibility)	NC	+	+				+		Zhang et al. (2014)
Protease			30 (feeding exp)									
Xylanase												
Protease	200 mg/Kg	Corn-soy	42	NC	+	+	–			–		Tactacan et al. (2016)
Xylanase	0.05 – 0.1 g/kg	Corn-soybean	42	NC	+	+	+			+		Lan et al. (2017b)
Protease	300,000 U/kg	Corn or sorghum	40	NC	+	+		+				Chen et al. (2017)
Xylanase	500 – 4000 U/kg	Wheat	28	NC	+	+				+		Dong et al. (2018)
Acid protease	1000 – 3000 U/kg	Corn-soybean	35	AB	ns	+					+	Han et al. (2017)
α-amylase	75 – 225 U/kg											
Xylanase	3500 – 5250 U/Kg											
β-mannanase	175 – 525 U/kg											
Glucose oxidase	60 – 180 U/kg											
Acid cellulose	60 – 180 U/kg											
Galactosidase	50 – 150 U/kg											
Bromelain	0.5 – 2 g/kg (given to sow)	Corn-soybean	28 (from 108 d gestation)	NC	+				+			Begum et al. (2015)

GP, Growth promoter; **ND, Nutrient digestibility**; FT/D, faecal traits/reduction of diarrhoea episodes; **GA/IS, Modulation gut architecture/promote immune response;** **GM, Gut microbiota modulator**; FG, fermentation products in the gut

Lopez-Galvez et al. 2021. Animal Feed Science and Technology 271: 114727

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Vitamins

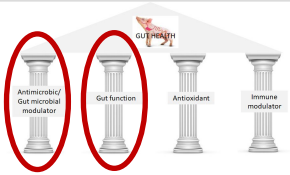
- Beyond their role as essential micronutrients, vitamins share several remarkable activities (antimicrobial, immunological and antioxidative).

- Vitamins can sustain gut health during the post-weaning through different mechanism.

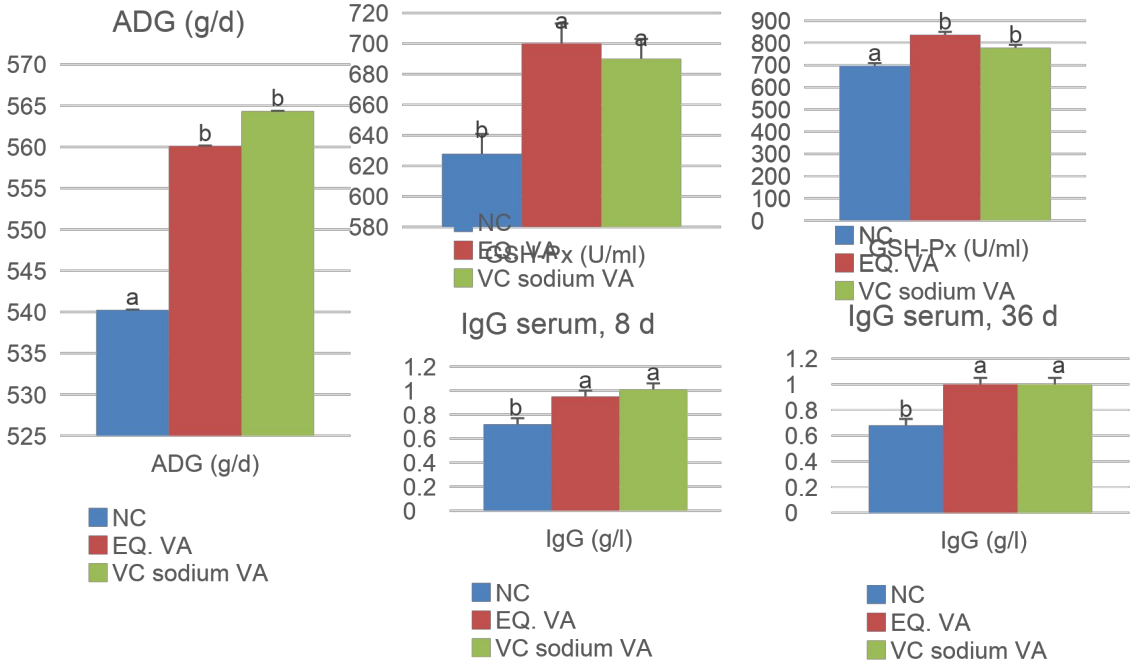
Adapted from *Lauridsen et al* (2021). *Animal Feed Science and Technology*, *273*, 114823.

Mechanism	Vitamin (or bioactive forms)	Impact
Inhibition of inflammation	B-vitamins, vitamin E	Inflamed gut appears to provide a favorable environment for ETEC
Control of oxidative stress	Vitamins with antioxidative activity	Prevention of excessive production of reactive oxygen species during an host-inflammatory induced response, and hence prevention of enteric infection.
Improvement of effective immune cell activity and response, and immune cell homeostasis	Vitamins E, C, A, D and B-complex	Formation of immune cells and their signals and modulation of immune cell responses.
Improvement of intestinal barrier function	Vitamins D and A	Regulation of tight junction molecules and hence prevention of barrier damage
Regulation and modulation of innate and adaptive immunity	Vitamins A and D	Gut homing, immune cell differentiation and cytokine suppression are important host responses to injury and infection and resolution of inflammation.
Production antimicrobial peptides	Vitamins A and D	Enhanced innate immunity and shaping of commensal microbiota
Shaping the microbiome	Thiamin, riboflavin, and vitamins A, B-6, B-12, C, D, E and K	Regeneration of commensal microbiota

Vitamin A



Vitamin A with L-ascorbic acid sodium salt improves the growth performance, immune function and antioxidant capacity of weaned pig



NC = basal diet without external Vitamin A; EQ VA = basal diet supplemented with 12 000 IU/kg Vitamin A with ethoxyquin; VC sodium VA = basal diet supplemented with 12 000 IU/kg Vitamin A with L-ascorbic acid

Zhou, H. B., et al. Animal 15.2 (2021): 100133.

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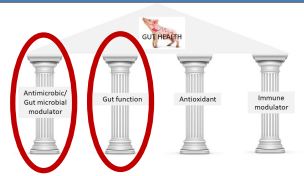
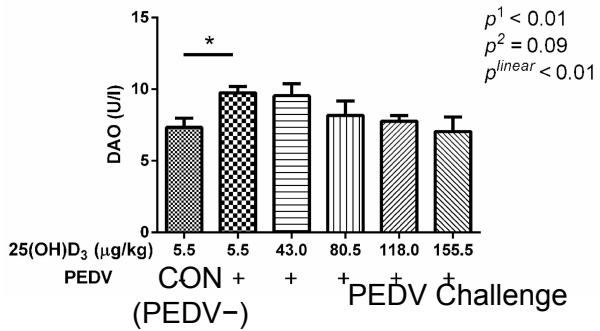
Vitamin D

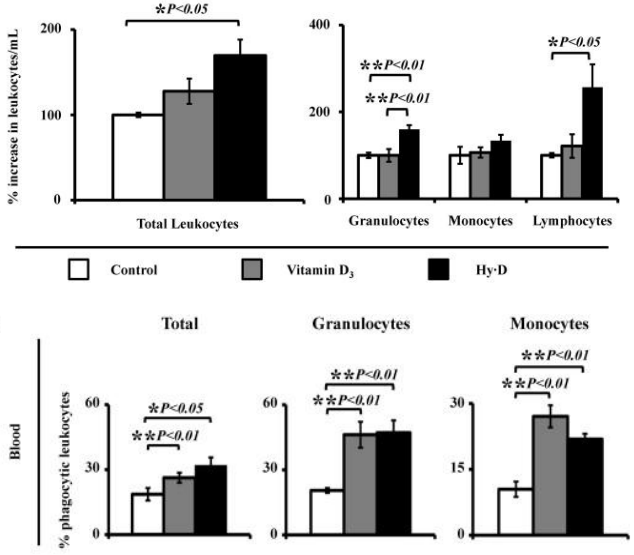


vitamin D3 could alleviate intestinal damage and protect against PEDV-induced inflammatory status.



25(OH)D_3 (μg/kg)	5.5	5.5	118	155.5	*p*
RIG1	1.00 ± 0.20	3.07 ± 0.68 *	1.94 ± 0.38	1.41 ± 0.30	0.02
TLR2	1.00 ± 0.14	3.94 ± 0.72 *,a	1.77 ± 0.54 b	1.58 ± 0.37 b	<0.01
MyD88	1.00 ± 0.10	1.42 ± 0.12 *	1.13 ± 0.12	1.16 ± 0.12	0.02
IFNλ	1.00 ± 0.23	2.06 ± 0.32 *,a	1.20 ± 0.25 b	1.10 ± 0.10 b	0.02
MxA	1.00 ± 0.24	2.96 ± 0.61 *,a	1.72 ± 0.17 a,b	1.05 ± 0.18 b	0.01
IL6	1.00 ± 0.20	2.49 ± 0.52 *,a	0.97 ± 0.28 b	0.71 ± 0.18 b	0.04
IL8	1.00 ± 0.20	2.73 ± 0.54 *,a	1.53 ± 0.50 a,b	0.95 ± 0.11 b	0.02

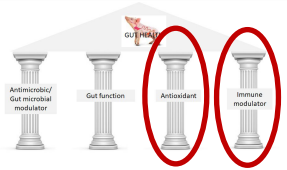
vitamin D3 increased of leukocyte numbers and increased their phagocytic capacity in weaned pigs



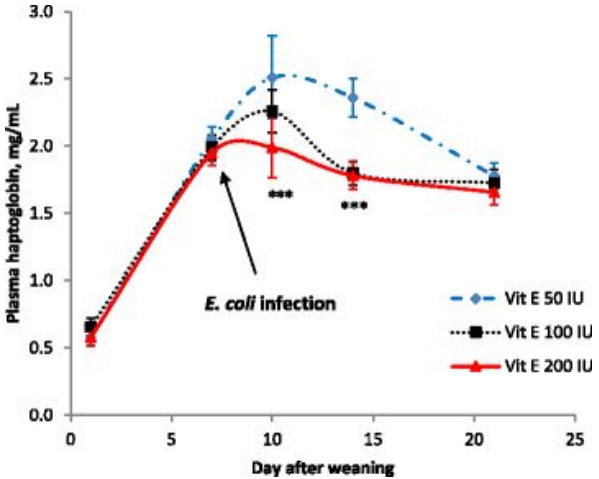
Vitamin D3= 3500 IU/kg ; Hy.D= 50 μg/kg 25(OH)D_3

Yang, J. et al., (2019). *Animals*, *9*(9), 627.
Konowalchuk et al. (2013). *Veterinary Immunology and Immunopathology*, *155*(1-2), 57-66.

Vitamin E

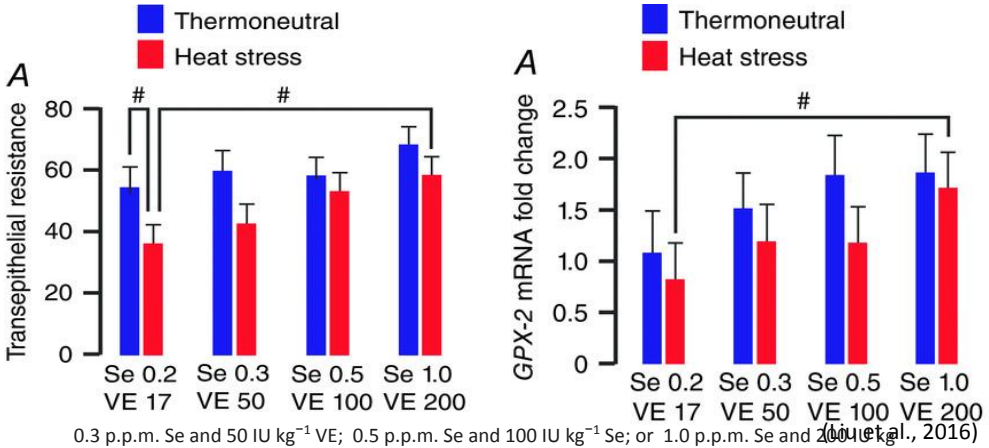


Effect of dietary vitamin E levels on plasma haptoglobin content after weaning



(*Kim et al.*,2016)

Selenium and vitamin E together improve intestinal epithelial barrier function and alleviate oxidative stress in heat-stressed pigs



0.3 p.p.m. Se and 50 IU kg^{-1} VE; 0.5 p.p.m. Se and 100 IU kg^{-1} Se; or 1.0 p.p.m. Se and 200 IU kg^{-1} (Liu et al., 2016)

Selenium and VE participate synergistically in neutralizing free radical

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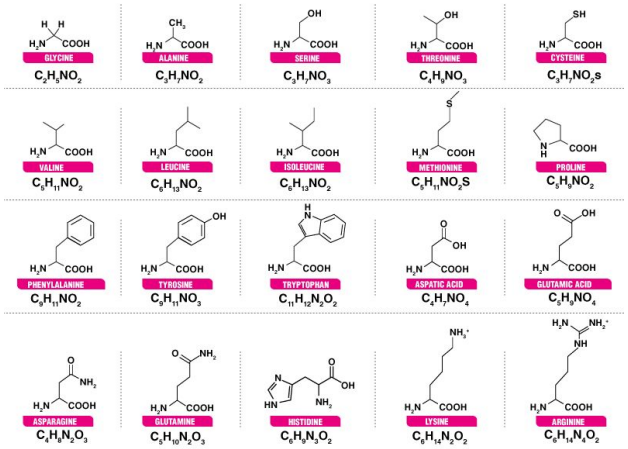
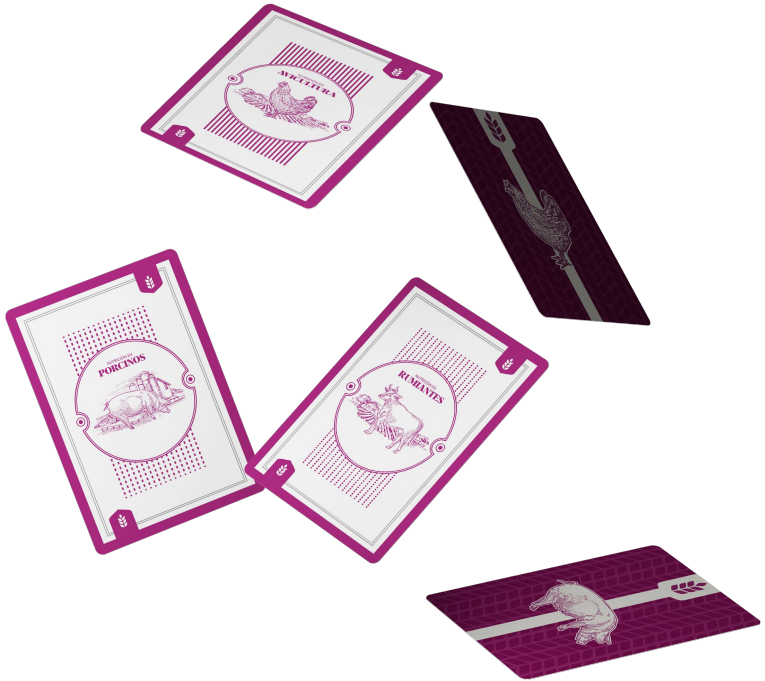
Kim et al., (2016) *J Animal Sci Biotechnol* **7,** 58; *Liu, et al.*, (2016). *Experimental physiology*, *101*(7), 801-810.

Amino acids


GLYCINE $C_2H_5NO_2$
ALANINE $C_3H_7NO_2$
SERINE $C_3H_7NO_3$
THREONINE $C_4H_9NO_3$
CYSTEINE $C_3H_7NO_2S$
VALINE $C_5H_{11}NO_2$
LEUCINE $C_6H_{13}NO_2$
ISOLEUCINE $C_6H_{13}NO_2$
METHIONINE $C_5H_{11}NO_2S$
PROLINE $C_5H_9NO_2$
PHENYLALANINE $C_9H_{11}NO_2$
TYROSINE $C_9H_{11}NO_3$
TRYPTOPHAN $C_{11}H_{12}N_2O_2$
ASPATIC ACID $C_4H_7NO_4$
GLUTAMIC ACID $C_5H_9NO_4$
ASPARAGINE $C_4H_8N_2O_3$
GLUTAMINE $C_5H_{10}N_2O_3$
HISTIDINE $C_6H_9N_3O_2$
LYSINE $C_6H_{14}N_2O_2$
ARGININE $C_6H_{14}N_4O_2$


AVICULTURA
PORCINOS
RUMIANTES

Amino Acids

Especially **glutamine, glutamate, arginine, aspartate and cysteine**, have been shown to cover three out of the four pillars, namely the gut function, antioxidant and immune mediators.

TABLE 1 | Amino acids influencing the indicators related to the 4 pillars of gut health in piglets.

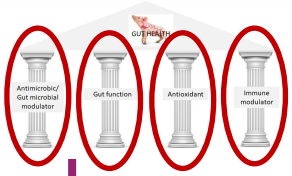
Pillars	Epithelial barrier and digestion		Immune fitness		Oxidative stress homeostasis		Microbiota balance	
Indicators of gut health	↗ Villus height ↗ Tight junctions ↗ Goblet cells and mucins ↗ Digestive enzymes activity ↗ Transporters ↗ Cell proliferation	↘ Diarrhea ↘ Permeability ↘ Cell apoptosis	↗ Immunoglobulins ↗ Anti-inflammatory cytokines	↘ Pro-inflammatory cytokines ↘ Lymphocytes proliferation	↗ Total Glutathione ↗ Antioxidative enzymes ↗ Anti-oxidative capacity	↘ Malondialdehyde ↘ Oxidized glutathione	↗ Diversity ↗ Beneficial bacteria (Lactobacillus, Bifidobacterium)	↘ Parasites (Eimeria) ↘ Harmful bacteria (Enterobacteria, Clostridium, Campylobacter)

Effect of amino acids	Support	Support and restore	Restore	Support	Support and restore	Restore	Support	Support and restore	Restore	Support	Support and restore	Restore
Asparagine	(56)	(57)		(56)								
Aspartate		(58)		(59, 60)				(61)		(59)		
Arginine	(62–67)	(68)	(69)	(62, 64)	(68)		(62, 65, 70, 71)					
Cysteine	(72)	(73)		(72)	(73)		(72)					
Glutamate or monosodium Glutamate	(67, 74–77)	(78)	(61)	(79)	(75, 78)		(77)	(78)	(61)			
Glutamine	(62, 80–89)	(90–93)	(94)	(62, 88)	(92, 93, 95)	(94, 96)	(88, 97)	(92)				
Isoleucine	(98)	(99)			(99, 100)							
Leucine		(101)										
Lysine	(102)	(103)								(102)		
Methionine	(104, 105)						(105)					
Proline	(106)											
Serine	(107)			(107)			(107)					
Threonine	(108, 109)			(110)								
Tryptophan	(111)	(42, 112–114)		(111)	(113, 115)		(116)	(114)				

↗, *increased in response to AA supplementation;* ↘, *decreased in response to AA supplementation.*

Chalvon-Demersay, et al. (2021). Frontiers in Veterinary Science, 8, 663727.

Glutamate and glutamine



	Energy	Cell proliferation	Immunity
Glutamate (Glu)	Good fuel for enterocytes		Precursor of glutathione in the intestine
Glutamine (Gln)	Provides energy to the immune cells in the gut	Precursor of glucosamine (involved in mucin synthesis) Activates mTOR phosphorylation	

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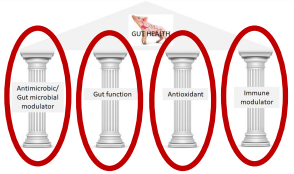
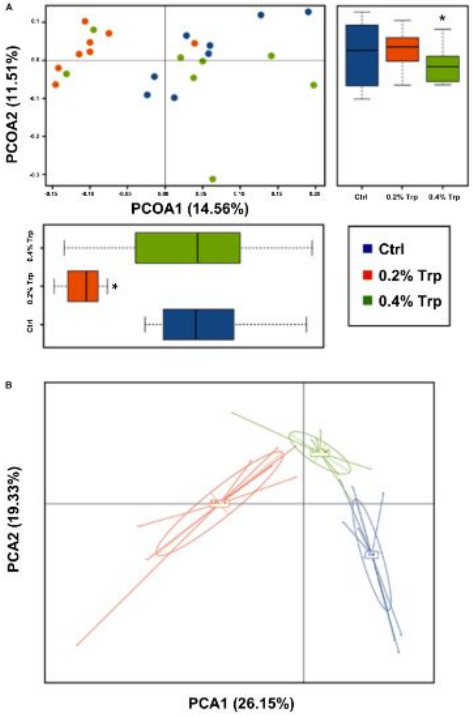
Amino acids and microbiota



Effect of Tryptophan inclusion in cecal microbiota of post weaning pigs



Liang et al., (2018). Frontiers in Microbiology, 9, 1736.

Effect of Glutamate and Glutamine inclusion in faecal microbiota of post weaning pigs

Comparison	baseMean [1]	log2FoldChange [2]	lfcSE [3]	P value [4]	P adj [5]	Taxa
Genus level						
100Glu vs CO	19.62	-21.65	2.78	<0.0001	<0.0001	Mogibacterium
	9.07	-21.07	5.28	<0.0002	0.007	Selenomonas
100Gln vs CO	34.79	-20.86	3.03	<0.0007	<0.0001	Fusobacterium
	8.33	-21.00	3.12	<0.0008	<0.0001	UBA1819, Family Ruminococcaceae

Luise et al., Scientific Reports, 12(1), 14533.

The effect of AAs supplementation as microbial modulators is still poorly studied.

However, some studies suggested that **glutamine, glutamate, tryptophan and arginine** can modulate the commensal bacterial profile of pigs, and contribute to the production of bioactive compounds including precursors for the production of the main SCFAs (isobutyrate, isovalerate, 2-methylbutyrate), indolic compounds (derived from tryptophan metabolism) and amines

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Considerations

- Early-weaning represent a multi factorial stress that in certain conditions significantly impair the gut health
- Compared to the antibiotic, that exert an antimicrobial effect, ZnO plays a multi layer role in prevent or restore the gut health impairment
- Feed additives, Feed material and Veterinary medicines represent part of the strategy to reduce the use of pharmacological dose of ZnO
- The 1:1 replacement of the therapeutic dose of the ZnO is not plausible
- The feeding strategies do not exert the same effect in all the conditions (e.g. sanitary status), important to understand the mechanism of action of the bioactive compounds to properly apply in target farms to maximize the results and save money.
- Revision of nutrient requirements to cope the physiological needs in post ZnO and post- antibiotic era is central

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