Evaluation of the efficacy of a carob seed product on the intestinal health of weaning piglets experimentally challenged with enterotoxigenic *Escherichia coli* (ETEC) K88

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Introduction

EARLY WEANING

Lactation ⟷ **Pre-starter**



Environment and mangement factors

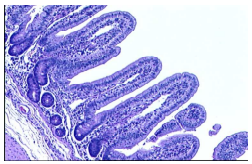
Stress



Anorexia | **Disbiosis** | **Inflamation** | **Translocation**







Colibacillosis

Enterotoxigenic *Escherichia coli* K88

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2006 UE ban Antibiotics as Growth Promoters (AGPs)

Search of new alternatives.



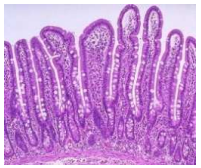
NEW OPPORTUNITIES FOR NEW FUNCTIONAL INGREDIENTES in pig production.

Introduction



Galactomanans

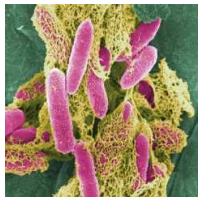
Immune response modulation
(Gallois *et al*., 2009)

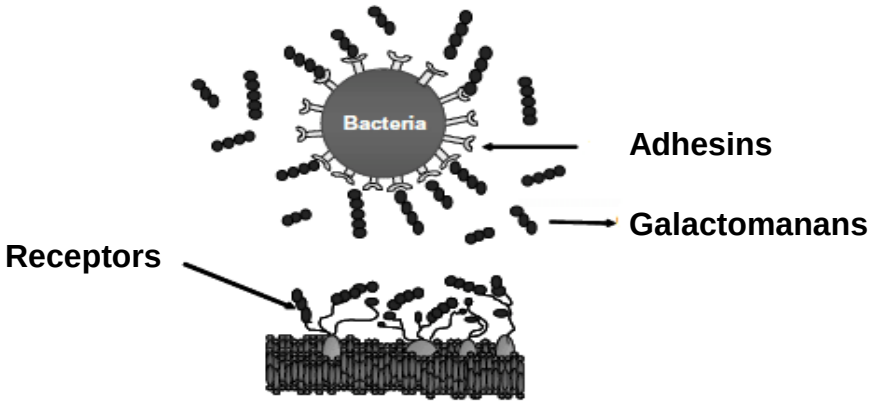


Prebiotics (Wang *et al*., 2010)

Blockage of microbial adhesion (Badía et al., 2012)

- Lactic bacteria
- Fermentation





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Introduction

The carob seed as a natural source of galactomanans

Fruit



(*Ceratonia siliqua*)

Seed



Carob seed

partially degermed, dehulled and micronized



Galactomanans (Manose/Galactose 4:1)

Other possible functional compounds (polyphenols…)

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Objetive

To evaluate the potential of a carob seed product, rich in galactomanans, in the prevention and/or control of post-weaning diarrhoea in piglets experimentally challenged with ETEC K88.

Animals & diets

Experimental Farms(UAB)

72 Weaning piglets(28 d)

24 pens (3 anim./pen)

3 boxes (8 pens/box)

n = 8

CTR: control diet

ZnO: CTR+ZnO (3 kg/Tm)

CRB: CTR + carob seed (5 kg/Tm)

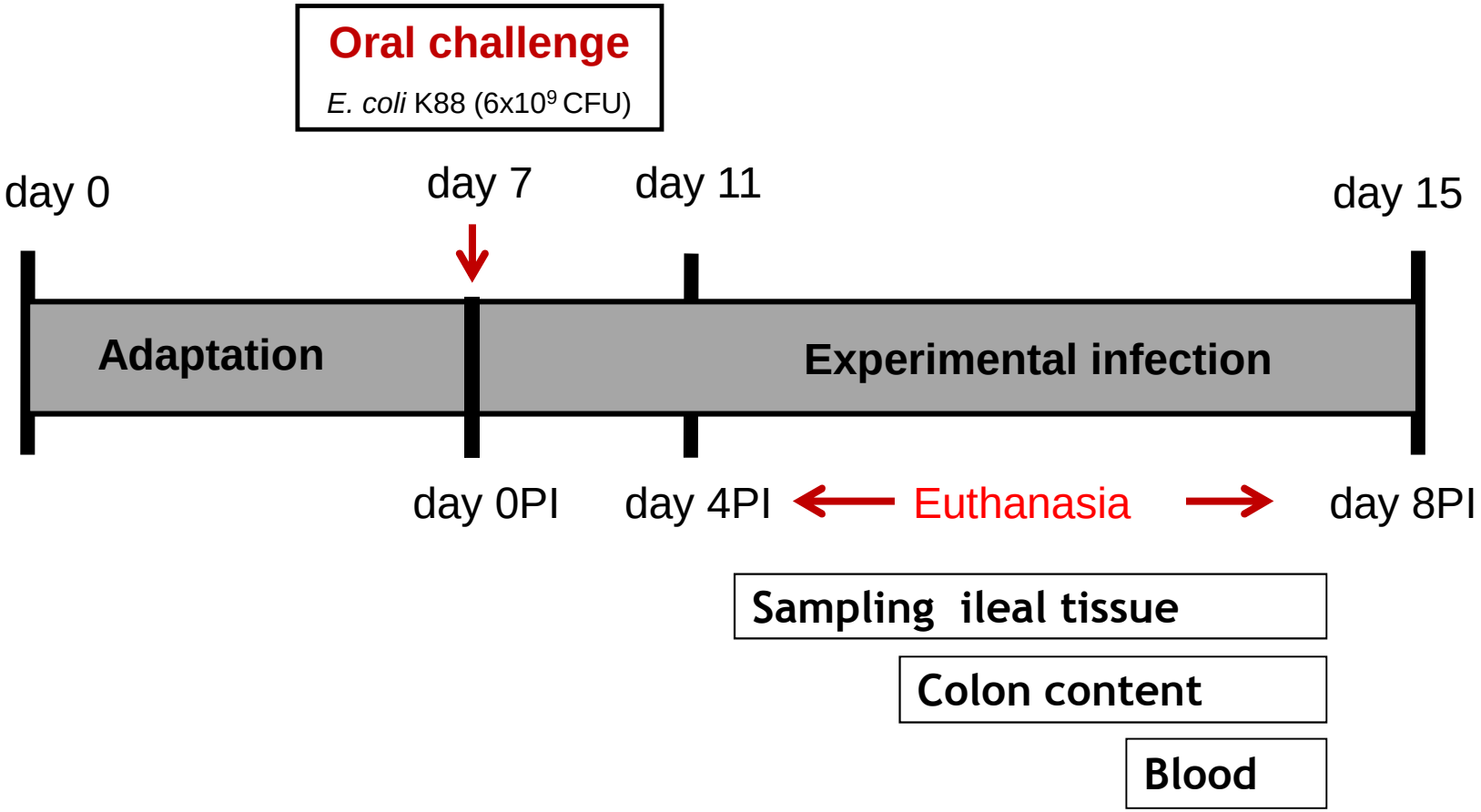




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Experimental design



Days 0, 7, 11 y 15: Weight recorders

Fecal samples (microbiology)

Analysis

Material & Methods

Faeces and ileal mucose: Traditional microbiology

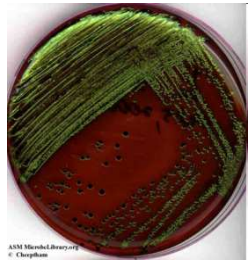
- **Enterobacteria**
- ***E. coli***

Colon digesta



qPCR

- **Enterobacteria**
- ***E.coli* K88**

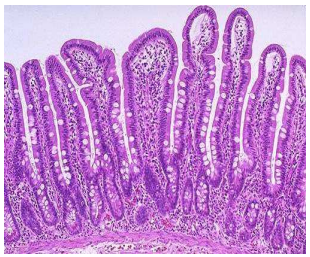


Ileal digesta **Viscosity**

Ileal & colon digesta **Fermentation products**

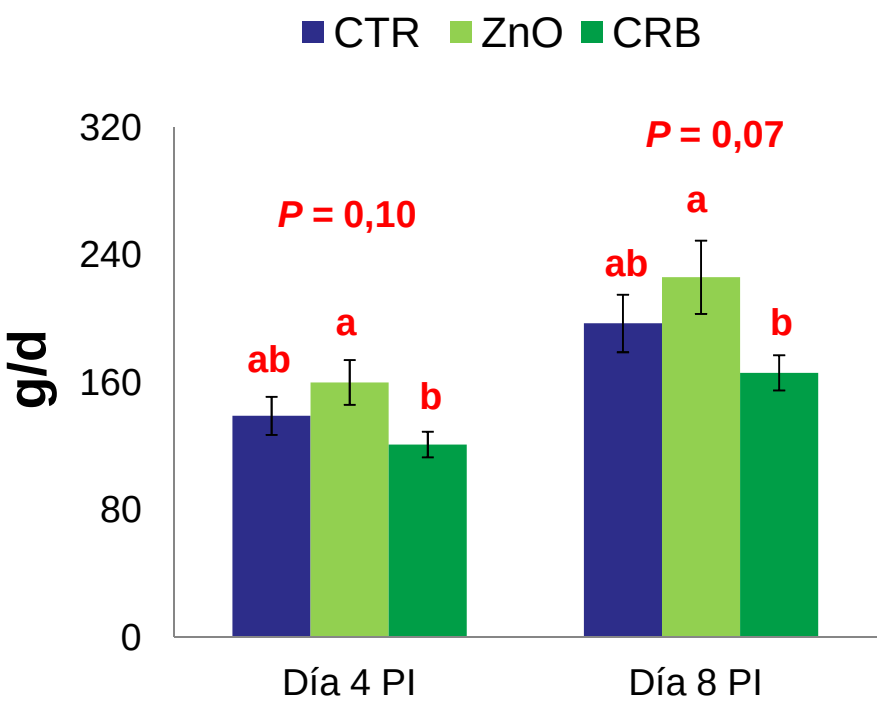
Blood: Acute Phase Proteins(Pig-Map); TNF-α

Ileal tissue: Histology

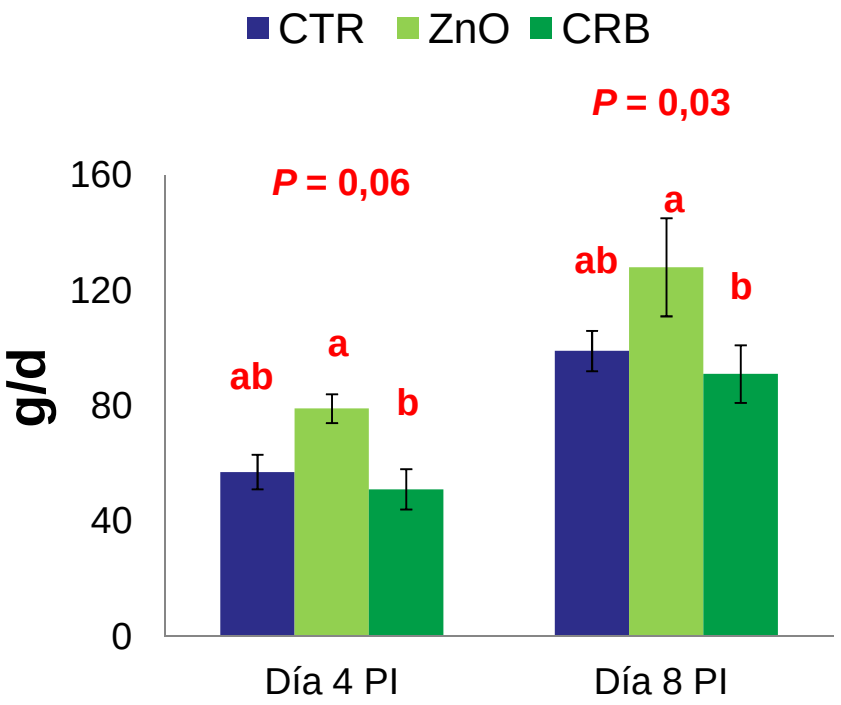


Performance

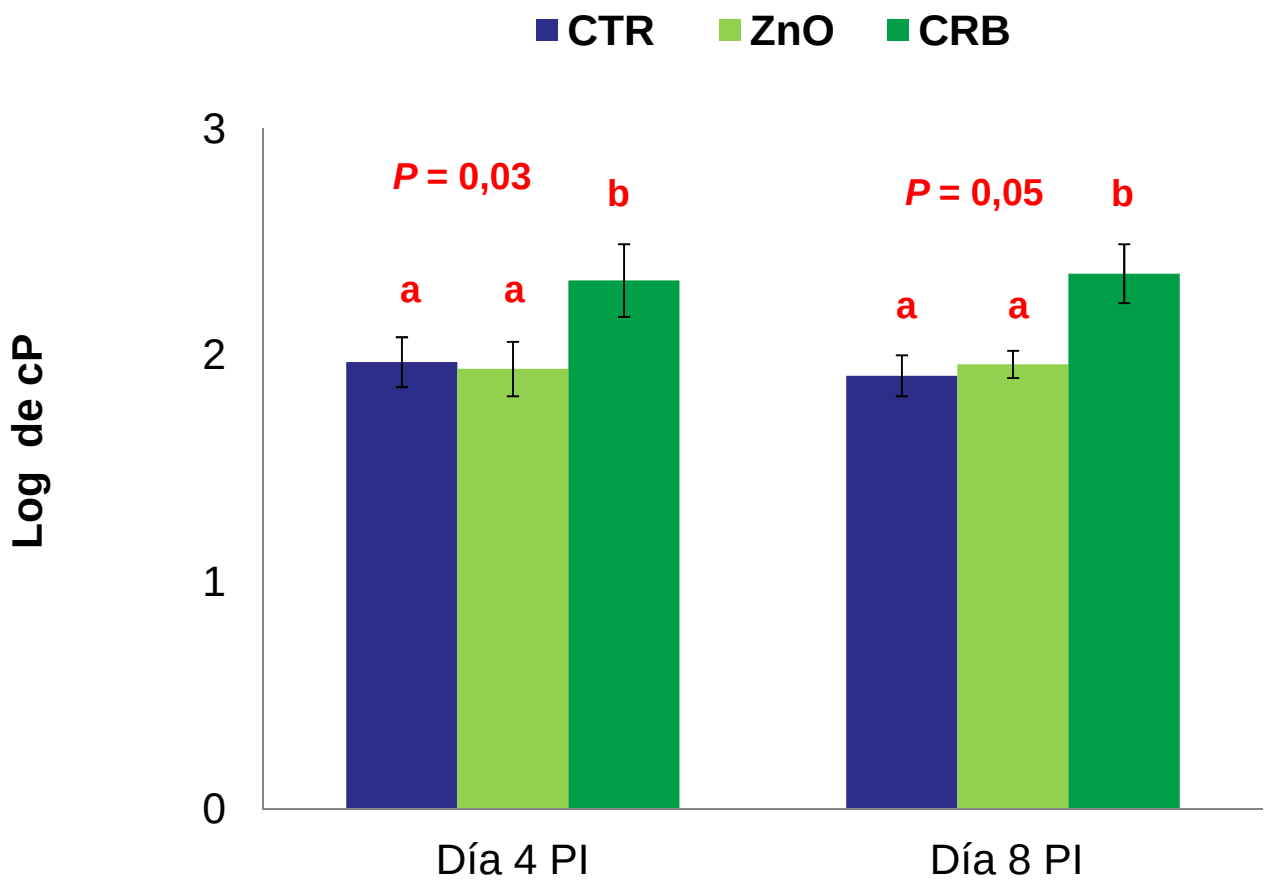
Feed intake



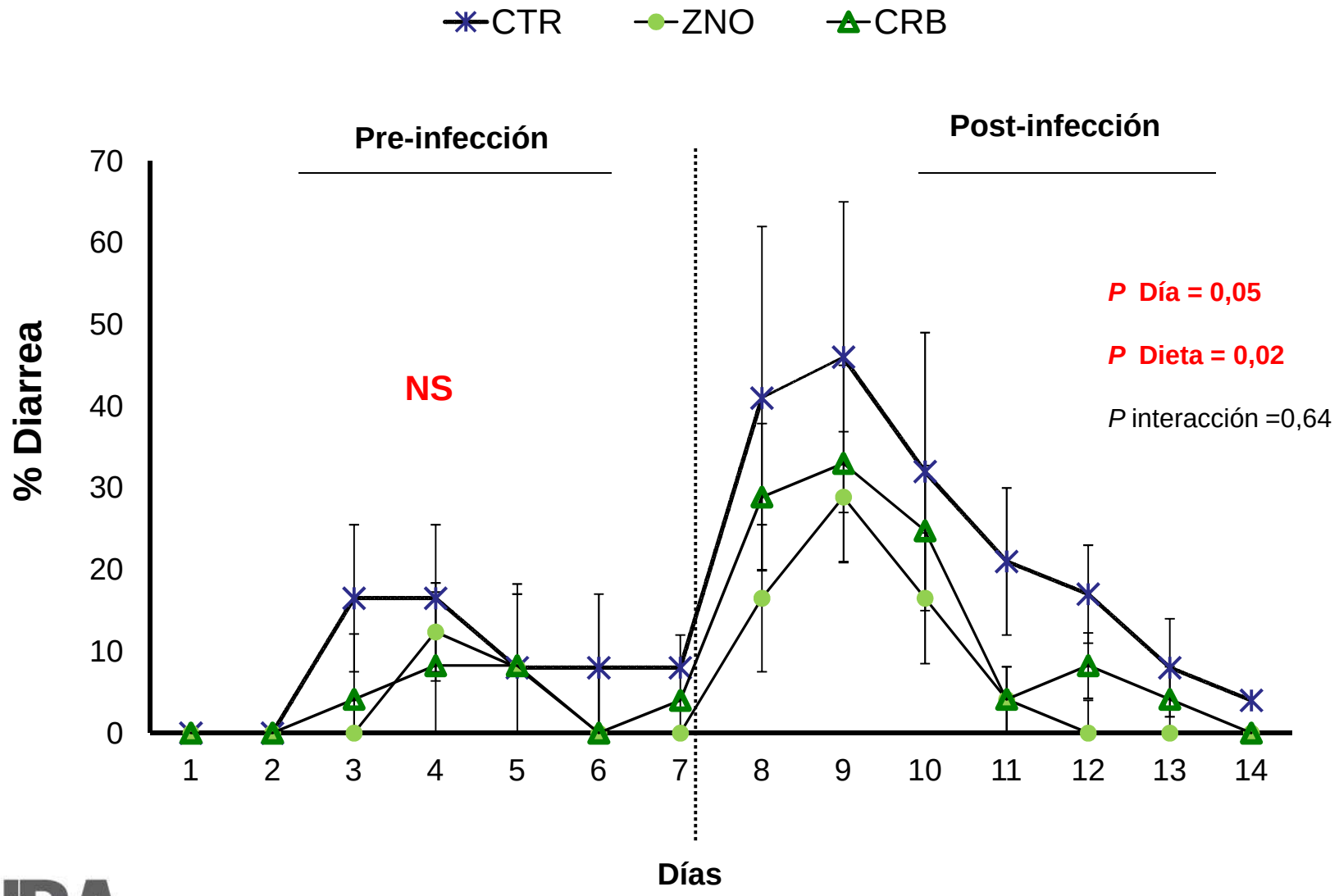
Weight gain



Ileal digesta viscosity



Diarrhoea incidence



Intestinal microbiota

Results

Faeces (CFU/gr)	Day PI	CTR	ZnO	CRB	P-value
Enterobacteria	0	9.5	9.3	9.7	0.56
	4	10.9^{y}	9.3^{x}	8.9^{x}	**0.05**
E. coli	0	9.0	9.2	9.0	0.65
	4	10.4^{y}	9.0^{x}	8.7^{x}	**0.03**

Colon (log gen copies/gr FM)					
Enterobacteria	4	11.9^{y}	9.1^{x}	9.7^{x}	**0.03**
***E. coli* - K88**	4	10.3^{y}	7.3^{x}	8.9^{xy}	**0.05**

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Adhered microbiota

Results

Ileal mucosa scrapes (CFU/gr)

	Day PI	CTR	ZnO	CRB	P-value
Enterobacteria	4	6.3 ^{y}	5.8^{y}	4.2^{x}	**0.09**
	8	5.4	5.5	5.0	0.99
E. coli	4	6.1 ^{y}	4.6^{x}	4.1 ^{x}	**0.07**
	8	5.9	5.3	5.0	0.96

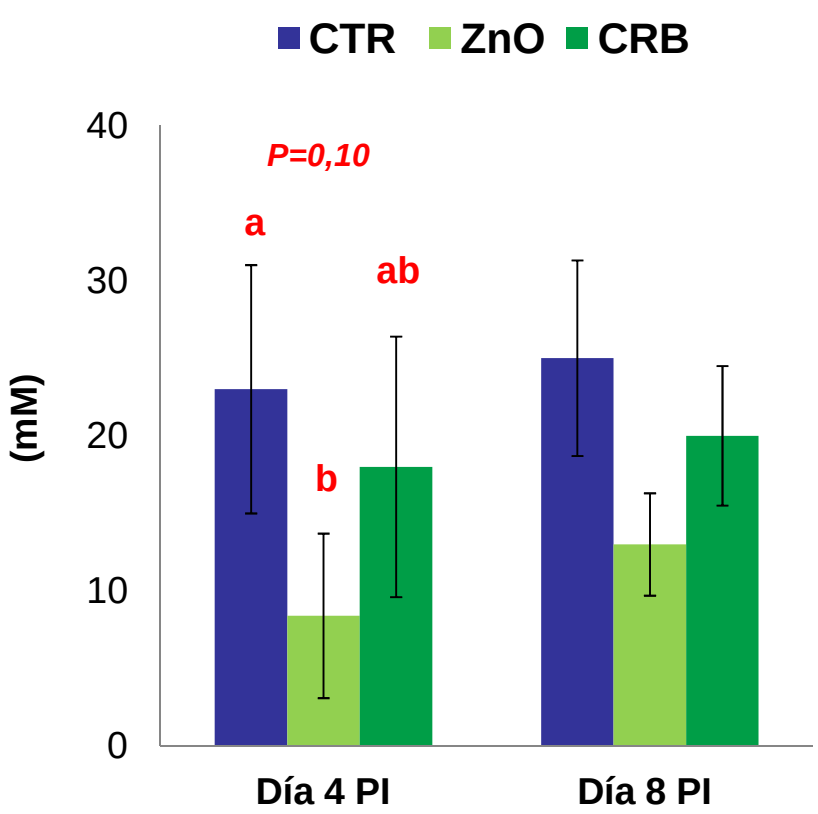
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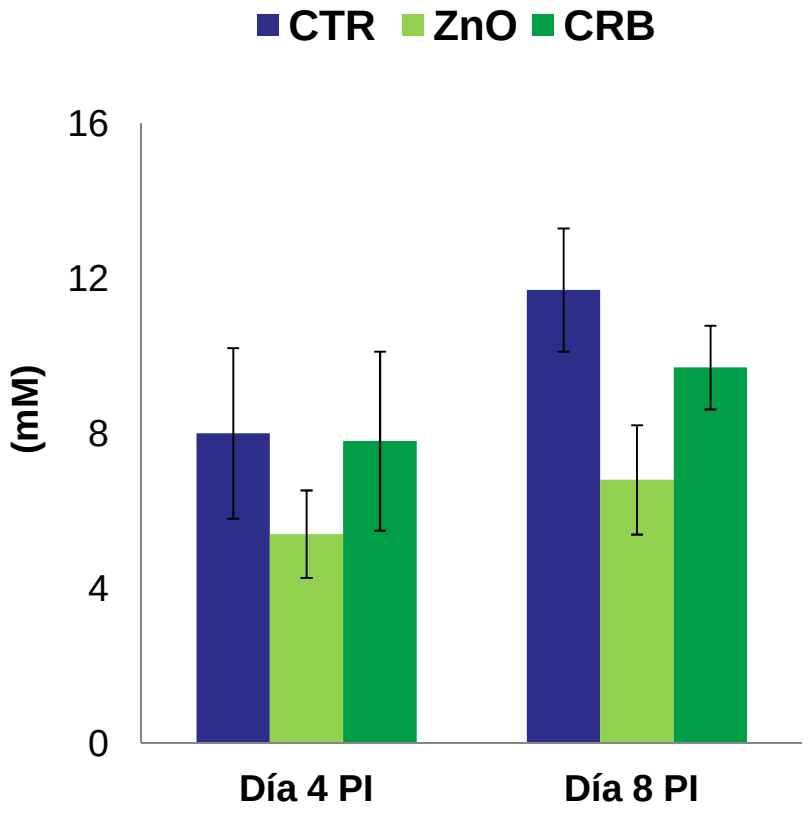
Fermentation products in ileon

Results

Lactic acid



Total SCFA



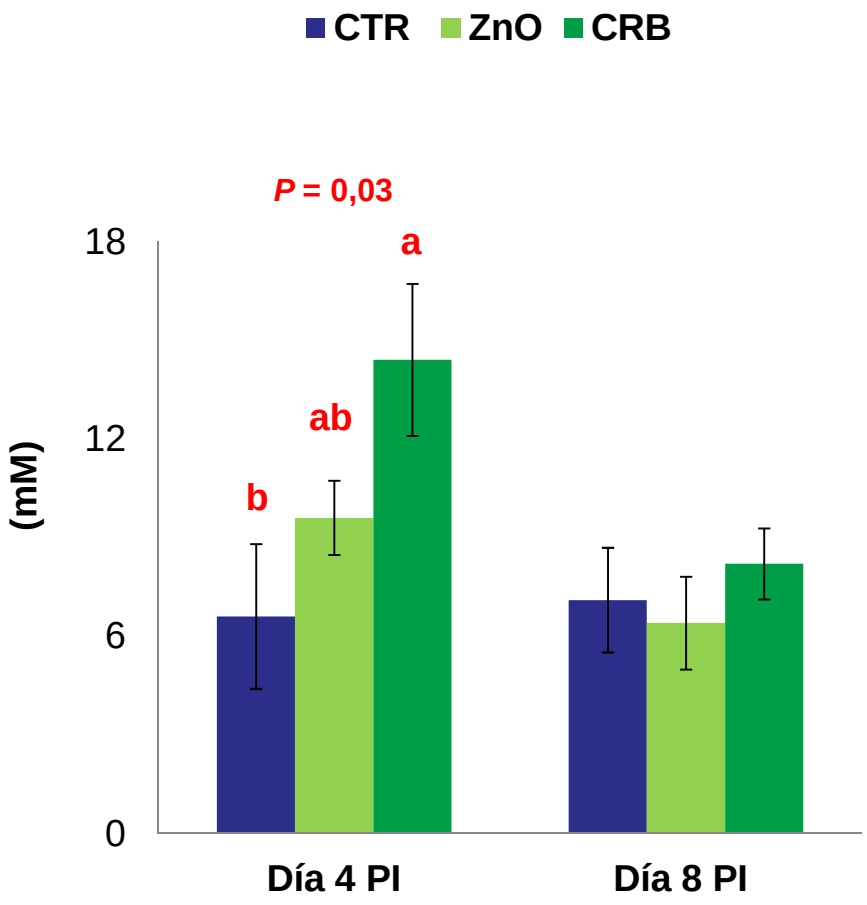
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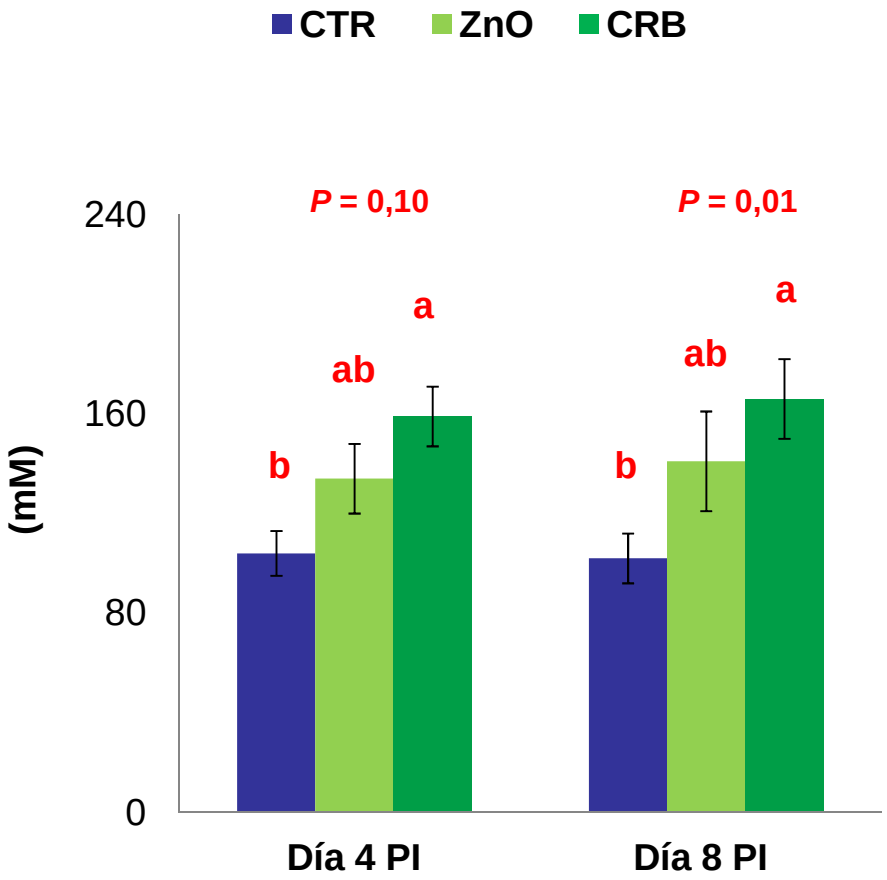
Fermentation products in colon

Results

Lactic acid



Total SCFA

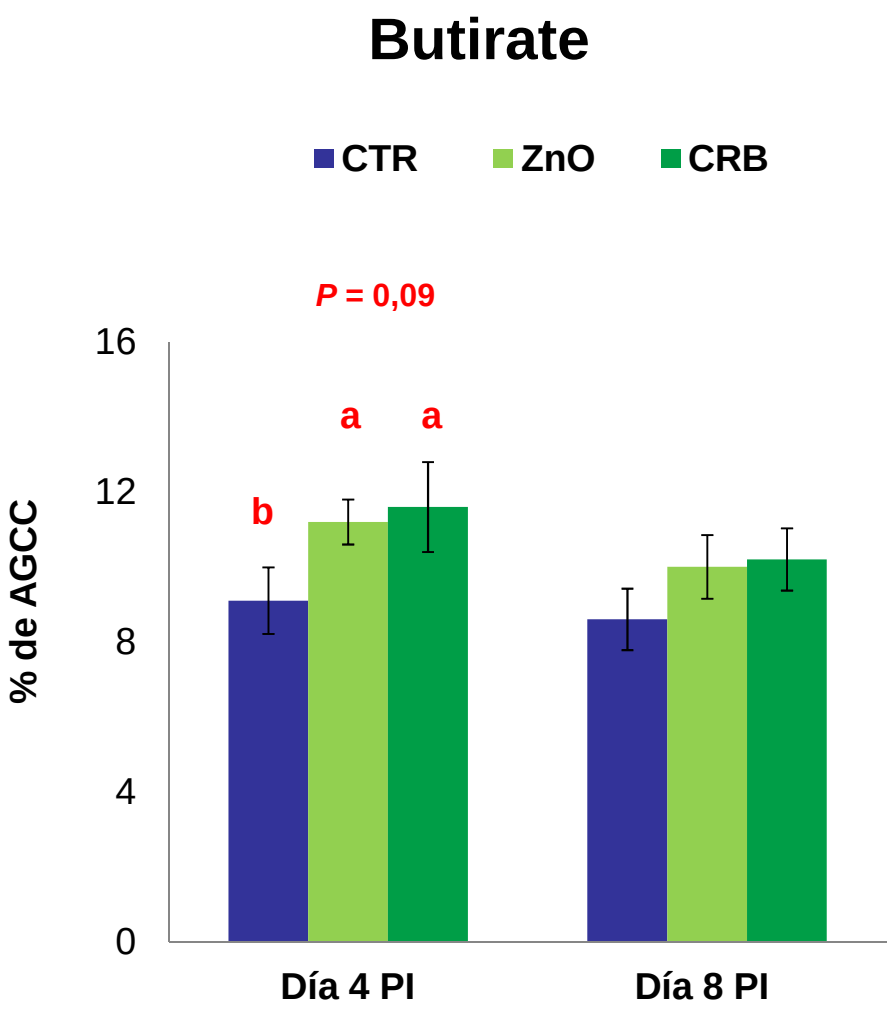


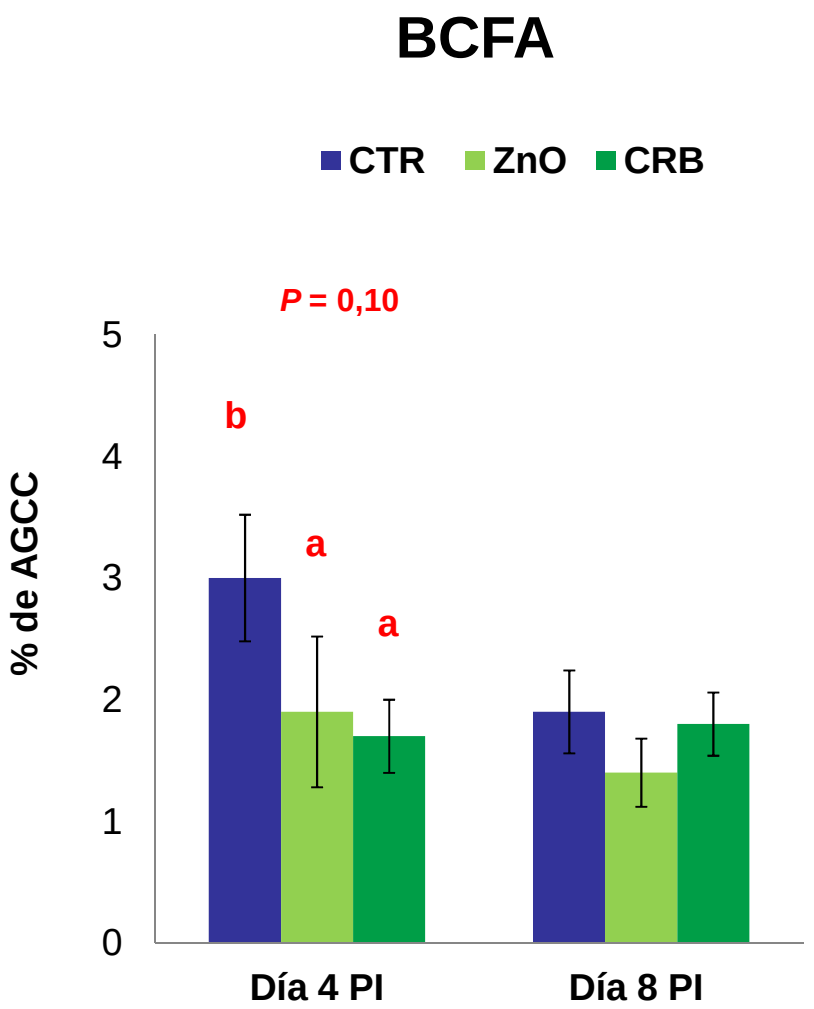
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Fermentation products in colon

Results





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Inflamation indexes

Results

Citokins TNF-α and Acute Phase Proteins PigMAP

	Day PI	CTR	ZnO	CRB	P-value
TNF-α (pg/mL)	4	68.4	67.6	66.2	0.99
	8	76.5	64.3	72.0	0.38
PigMAP (mg/dL)	4	1.20^{y}	0.70^{x}	0.74^{x}	**0.03**
	8	1.37^{y}	0.53^{x}	0.83^{xy}	**0.03**

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Histology

Results

	Day PI	CTR	ZnO	CRB	P-value
Villi height	4	308	323	321	0.20
(µm)	8	322^{xy}	349^{x}	317^{y}	**0.01**
Cripth depth	4	211	215	201	0.81
(µm)	8	207	197	206	0.57
IE lymphocites	4	9.2^{y}	7.0^{x}	7.9^{x}	**0.05**
(/100 µm)	8	10.0	9.0	8.7	0.18
Goblet cells	4	6.8^{y}	8.2^{x}	8.9^{x}	**0.04**
(/100 enterocites)	8	8.8	9.6	9.1	0.15

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Discussion

- Our results show the ability of this carob seed product to **reduce the enterobacteria and the ETEC K-88 proliferation** in the intestine of pigs experimentally challenged.

- This effect could be due to a **prebiotic effect** with a reinforcement of the indigenous microbiota. It is observed an increase in the fermentative activity in the colon with a **more butyrogen and less proteolytic fermentation**.

- It must be consider a possible **interference on the microbial adhesion mechanisms**. In that sense it is observed a decrease in the number of enterobacterias and coliforms adhered to the ileal mucosa together with **increases in the number of goblet cells**.

- A reduced exposition to the pathogen could explain the observed **reduction in the inflammatory response** (IEL; Pig-MAP).

CONCLUSION

The carob seed product assayed in this study at 5 kg/Tm, shows a clear potential in the prevention of the post-weaning colibacillosis induced by ETEC K-88.

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