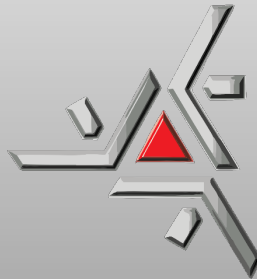


Intestinal barrier function of piglets fed AGP free diets supplemented with an association of fatty acids, prebiotics and zinc proteinate

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Introduction



Increased concern surrounding bacterial resistance to AGPs used in pig production diets.

Post weaning piglets

Some compounds may act improving the intestinal barrier, like some fatty acids, prebiotics and zinc proteinate.

The association of these compounds may provide a better result than each one individually added in piglet diets.

Objectives



To evaluate the association of fatty acids, prebiotics and zinc proteinate in piglet diets (21- to 49- d old) without AGP, on tight junction gene expression and microorganism translocation to the liver.

Material and methods



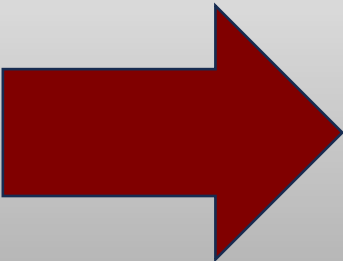
Animals:

- Eighty weaned piglets averaging 6.69 ± 0.19 kg
- Age of weaning: 21 days

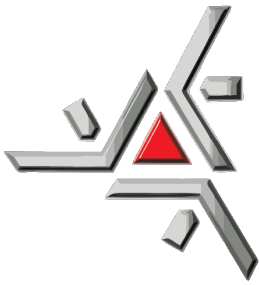
Experimental design:

- Randomized blocks design
- Five treatments
- Eight replicates - one male and one female

Treatments:

- Positive Control – PC (0.02% Halquinol 60%)
 - 0.00% FPZ
 - 0.10% FPZ
 - 0.14% FPZ
 - 0.18% FPZ
- 
- FPZ - Sodium Butirate,
Prebiotic and zinc
proteinate (Viligen NE)

Material and methods



- Gene expression of intestinal tight junctions

Table 1. Sequence of primers used in qPCR reactions

Genes	Gene sequence (5'→3')	Amplicon (pb)	Acess number	[cDNA]
ZO-1	F: GAGGATGGTCACACCGTGGT R: GGAGGATGCTGTTGTCTCGG	169	AJ318101.1	80 ng/μl
TJP2	F:ATTCGGACCCATAGCAGACATAG R:GCGTCTCTTGGTTCTGTTTTAGC	90	NM_001206404.1	80 ng/μl
OCN	F: ATGCTTTCTCAGCCAGCGTA R: AAGGTTCCATAGCCTCGGTC	176	NM_001163647.2	40 ng/μl
CLDN2	F: GGTGACATCTAGCGCCATCT R: AAGAAGACTCCACCCACCAC	123	NM_001161638.1	80 ng/μl
CLDN3	F: CCTACGACCGCAAGGACTAC R: GACTGGTCTCGGATGCAAGG	111	NM_001160075.1	80 ng/μl
CLDN4	F: CGTACCGACAAGCCCTACTC R: GCAGTCCAGGGAGAAACCAA	119	NM_001161637.1	40 ng/μl
CLDN5	F: CCTTCCTGGACCACAACATC R: CACCGAGTCGTACACCTTGC	110	NM_001161636.1	120ng/μl
β-actina	F: GGTTCATTTTGAGTGGGCAGC R:CTGGTCTCAAGTCAGTGTACAG	137	XM_003124280.3	40 ng/μl

ZO-1: zonula occludens 1 (Zhou et al., 2014), TJP2: tight junction protein 2 (Zhang et al., 2016), OCLN: occludin (Zhou et al., 2014), CLDN2: claudin-2 (Liu et al., 2017), CLDN3: claudin-3 (Liu et al., 2017), CLDN4: claudin-4 (Liu et al., 2017) and CLDN5: claudin-5 (Zong et al., 2019). The annealing temperature for all primers was 60°C. 2bp: base pairs. F: forward; R: reverse.

Material and methods



- **Translocation of bacteria to the liver**
 - Liver samples ($\pm 10\text{g}$) diluted with a phosphate buffer solution (pH 7.2).
 - Plates aerobically incubated at $37^{\circ}\text{C}/24$ hours
 - MRS and VRB agar for *Lactobacillus spp* and *Enterobacteriaceae*
- **Statistical analyzes**
 - Analysis of variance (ANOVA) and Dunnett's test to compare PC to each treatment
 - Polynomial regression and/or Linear Response Plateau (LRP) for dietary FPZ levels.

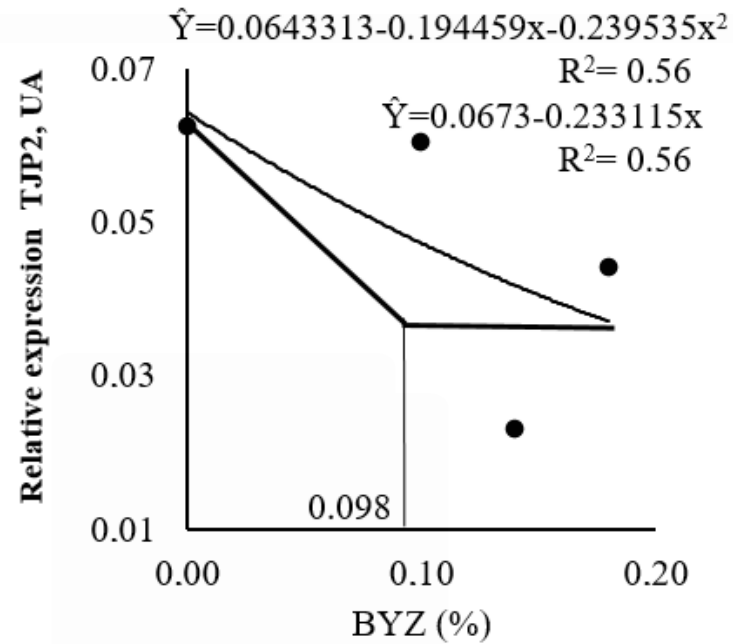
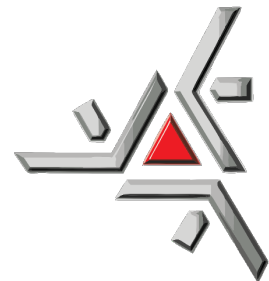
Results



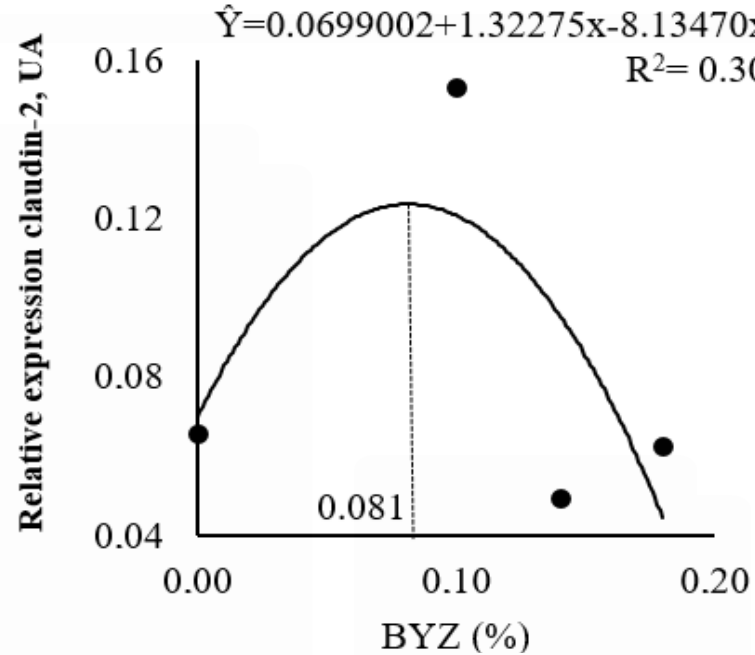
Table 2. Tight junctions' gene expression of the jejunum of 49-day-old piglets fed diets with antibiotic growth-promoter (PC) and association of sodium butyrate, dehydrated hydrolyzed yeast and zinc proteinate (FPZ) from weaning (21 days of age) to 49 days of age, expressed as UA

Item	PC	FPZ, %				SD	P-value
		0.00	0.10	0.14	0.18		
21- 49 dias							
ZO-1	0.01923 ^a	0.01063 ^b	0.01259 ^b	0.01406 ^a	0.01234 ^b	0.004	0.0205
TJP2	0.07245 ^a	0.06266 ^a	0.06045 ^a	0.02299 ^b	0.04440 ^b	0.010	0.0001
OCLN	0.03927	0.02755	0.03210	0.05544	0.04017	0.019	0.1397
CLDN2	0.19675 ^a	0.06578 ^b	0.15327 ^b	0.04930 ^b	0.06245 ^b	0.022	0.0001
CLDN3	0.01587 ^a	0.00765 ^b	0.01316 ^a	0.00688 ^b	0.00724 ^b	0.003	0.0008
CLDN4	0.03593	0.02461	0.02902	0.02660	0.03852	0.009	0.0538
CLDN5	0.00234 ^a	0.00047 ^b	0.00163 ^b	0.00068 ^b	0.00023 ^b	0.000	0.0001
Item	Equations					R ²	Model
TJP2	$\hat{Y}=0.063736-0.153431x$					0.42	L
	$\hat{Y}=0.0643313-0.194459x+0.239535x^2$					0.56	Q
	$\hat{Y}=0.0673-0.233115x$					0.56	LRP
CLDN2	$\hat{Y}=0.0901143-0.0706035x$					0.10	L
	$\hat{Y}=0.0699002+1.32275x-8.13470x^2$					0.30	Q
CLDN3	$\hat{Y}=0.00923609-0.00476518x$					0.11	L
	$\hat{Y}=0.00789494+0.876799x-0.539715x^2$					0.36	Q
CLDN5	$\hat{Y}=0.000846553-0.000923046x$					0.12	L
	$\hat{Y}=0.00497131+0.0233693x-0.141824x^2$					0.70	Q

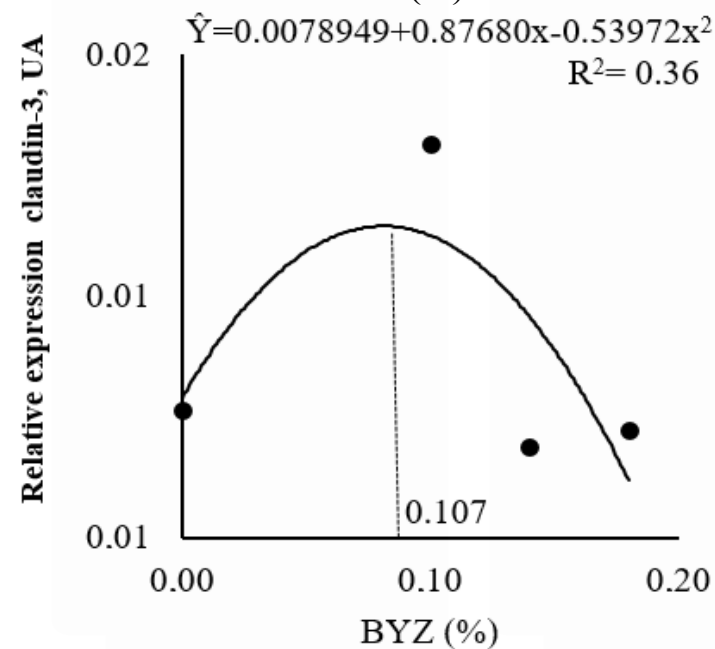
Different superscript letters on the row differ by Dunnett's test; PC = positive control (0.02% Halquinol - 60%); L= linear; Q = quadratic; LRP = linear response plateau;



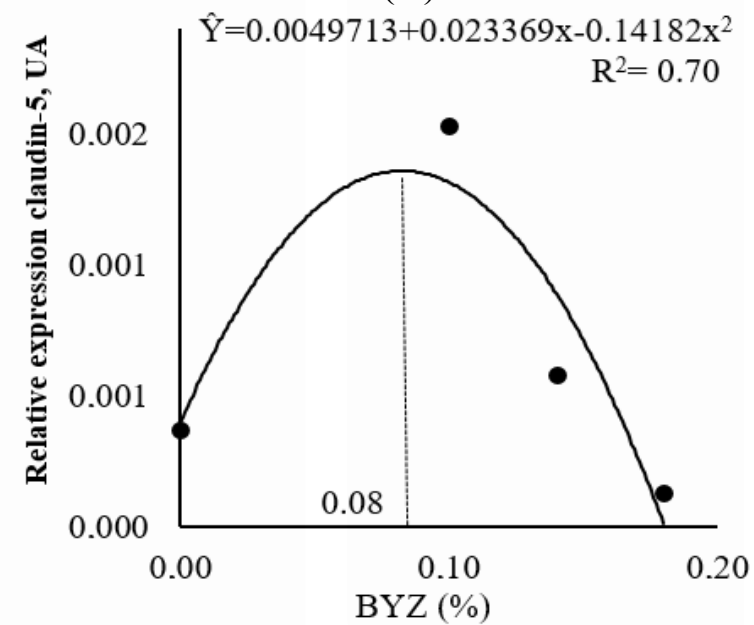
(A)



(B)



(C)



(D)



Table 3. Translocation of microorganisms to the liver of piglets fed diets with antibiotic growth-promoter (PC) and association of sodium butyrate, dehydrated hydrolyzed yeast and zinc proteinate (BYZ) from weaning (21 days of age) to 49 days of age (CFU, Log₁₀)

Item	PC	BYZ, %				SD	P-value
		0.00	0.10	0.14	0.18		
<i>Enterobacteria spp</i>	2.64	2.67	2.64	2.68	2.54	0.55	0.9554
<i>Lactobacillus spp</i>	2.63	2.39	2.79	2.76	2.72	0.53	0.2236

Different superscript letters on the row differ by Dunnett's test; CFU = colony-forming unit; PC = positive control (0.02% Halquinol - 60%); SD = standard deviation.

Conclusion



Supplementing about 0.10% of an association of fatty acids, prebiotics and zinc proteinate in piglet diets (21- to 49- d old), without AGP, was effective to maintain the function of intestinal barrier.

Thank you!

