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"Animal nutrition and nutrigenomics"

Phytobiotic effect of *Anacardium occidentale* powder on performance, gut health, diarrheal syndrome and anti-inflammatory and immune response of weaned piglets

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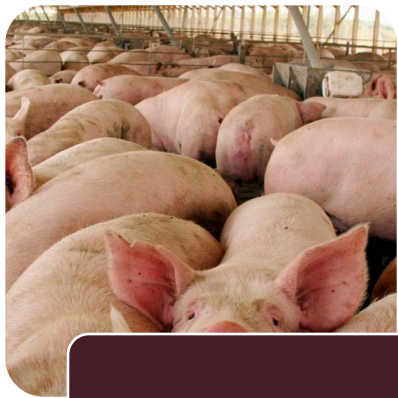
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INTRODUCTION

Intensive Animal Production System



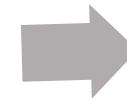
Pig Production



Dysbiosis

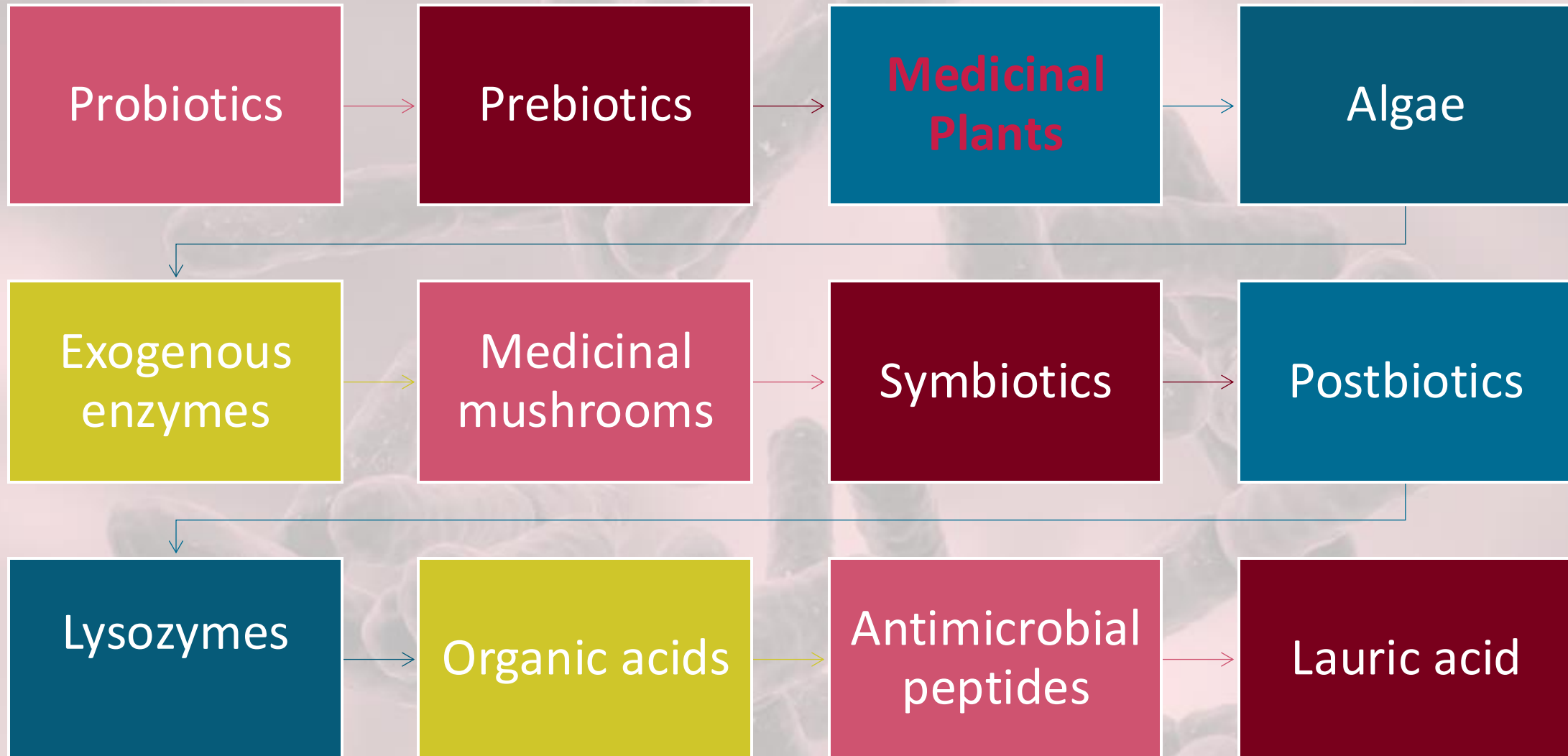


Antibiotic growth promoters (AGPs)



Resistant strains

Alternatives to AGPs



Phytogenic additives

Anacardium occidentale



Anti-inflammatory, antibacterial, analgesic, anticoagulant, antispasmodic and astringent properties

To evaluate the effect of dietary supplementation with *Anacardium occidentale powder* on the growth performance, diarrhea incidence, blood biochemistry, and intestinal traits of weaned piglets.

General
objective



Materials and Methods



4 groups (Control and 0,5, 1 and 1.5% of AOLP) in 72 piglets ([Yorkshire × Landrace] × Duroc) with 28 days

6 replications/group, 3 animals/replication

BW



Day 0

BW
ADG



Day 14

BW
ADG



Day 28

ANOVA
unidirectional

Tukey for
multiple tests

Day 28

Plasma samples for antioxidant indices and immunoglobulin content

3 cm of duodenum, jejunum, and ileum for morphology

Mucosa from jejunum and ileum for RNA extraction

Fecal samples for digestibility, VFA and Microbiota Diversity

T-Test for independent samples

Diarrhea incidence according to fecal scores assessed daily/animal

Five-point fecal consistency scoring system:

1 = hard, dry stools

2 = firm, formed stool

3 = soft, moist stools that retain their shape

4 = loose, shapeless stools

5 = aqueous liquid that can be poured

Stools of liquid consistency (score 4 and 5) were considered diarrhea

$$DI = \frac{\text{Number of diarrheas}}{\text{Animal number} \times \text{Total Days}} \times 100$$

Chi-square

The differences were considered statistically significant at $P \leq 0.05$, while the treatment effect was identified as a trend at $0.05 < P \leq 0.10$

Previous results

Secondary metabolite profile of *A. occidentale* powder

Compounds		Mean (n=3)	SD ($\pm$)	CV (%)
Quercetin	3-O-glucoside-7-O-rhamnoside	0.54	0.025	4.630
Chicoric acid		0.62	0.037	5.968
Kaempferol-7-O-glucoside		1.95	0.115	5.897
Quercetin		10.25	0.544	5.307
Caffeic acid		0.22	0.008	3.636
Cinnamic acid		0.25	0.012	4.848

Results

Table 3. Effect of dietary supplementation with AOLP on growth performance of weaned piglets.

Experimental Treatments								
Items	T0	T1	T2	T3	SEM	p-Value		
						ANOVA	Linear	Quadratic
Body Weight (kg)								
Day 0	8.03	8.03	8.03	8.03	0.31	1.000	0.953	0.988
Day 14	10.72	10.68	10.50	10.59	0.32	0.952	0.666	0.835
Day 28	14.19	14.32	14.16	15.46	0.52	0.458	0.230	0.384
Average Daily Gain (g)								
Days 0–14	192	189	177	183	9.59	0.951	0.655	0.838
Days 14–28	248	260	261	348	24.54	0.478	0.195	0.461
Days 0–28	220	225	219	265	11.41	0.441	0.222	0.372
Average Daily Feed Intake (g)								
Days 0–14	267	299	261	251	10.38	0.484	0.417	0.354
Days 14–28	449	495	474	598	28.39	0.147	0.054	0.404
Days 0–28	358	397	368	425	13.99	0.233	0.132	0.720
Gain–Feed Intake Ratio								
Days 0–14	0.72	0.63	0.65	0.73	0.02	0.096	0.722	0.017
Days 14–28	0.46	0.51	0.55	0.58	0.03	0.717	0.266	0.869
Days 0–28	0.60	0.57	0.60	0.63	0.02	0.793	0.511	0.497

T0: control; T1: 5 g/kg of AOLP; T2: 10 g/kg of AOLP; T3: 15 g/kg of AOLP. SEM = standard error of the mean.

Table 4. Effect of dietary supplementation with AOLP on diarrhea incidence of weaned piglets (%).

Experimental Treatments					
Items	T0	T1	T2	T3	p-Value
Days 0–14	6.35	5.16	5.15	9.92	0.106
Days 14–28	18.47 ^a	8.33 ^b	9.35 ^b	5.60 ^b	<0.001
Days 0–28	12.63 ^a	7.00 ^b	7.50 ^b	8.16 ^b	0.007

^{a,b}: mean values with different superscript letters within a row were significantly different ($p < 0.05$). T0: control; T1: 5 g/kg of AOLP; T2: 10 g/kg of AOLP; T3: 15 g/kg of AOLP.

Antidiarrheal properties

Results

Figure 1. Effect of dietary supplementation with AOLP on plasma antioxidant indexes of weaned piglets on day 14 (A) and day 28 (B). Results are presented as mean $\pm$ SEM; $n = 6$. * $p < 0.05$ indicates difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP; CAT = catalase; SOD = superoxide dismutase; GSH-Px = glutathione peroxidase; MDA = malondialdehyde; and T-AOC = total antioxidant capacity.

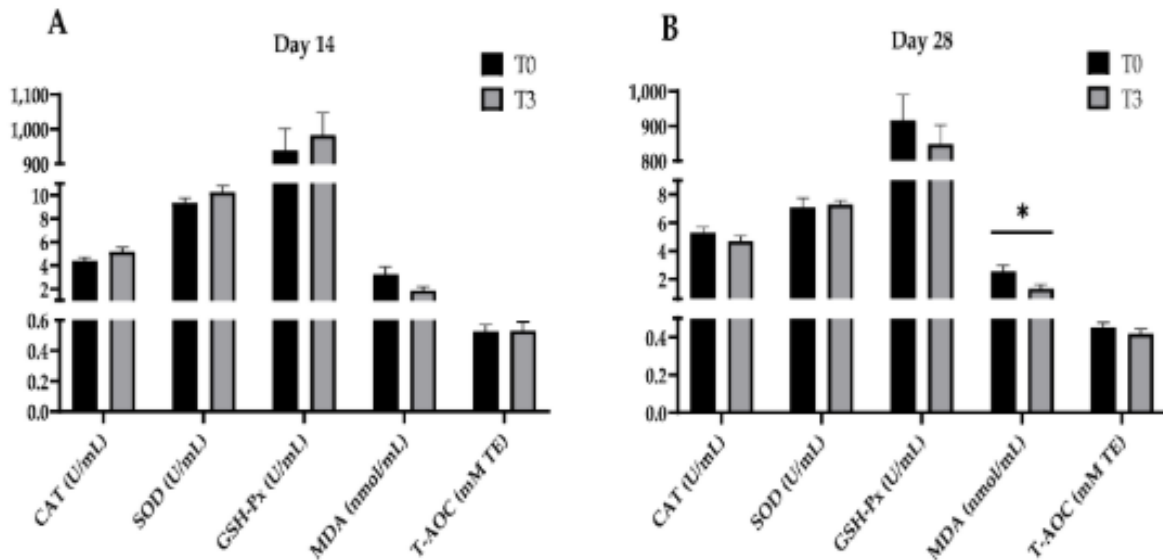
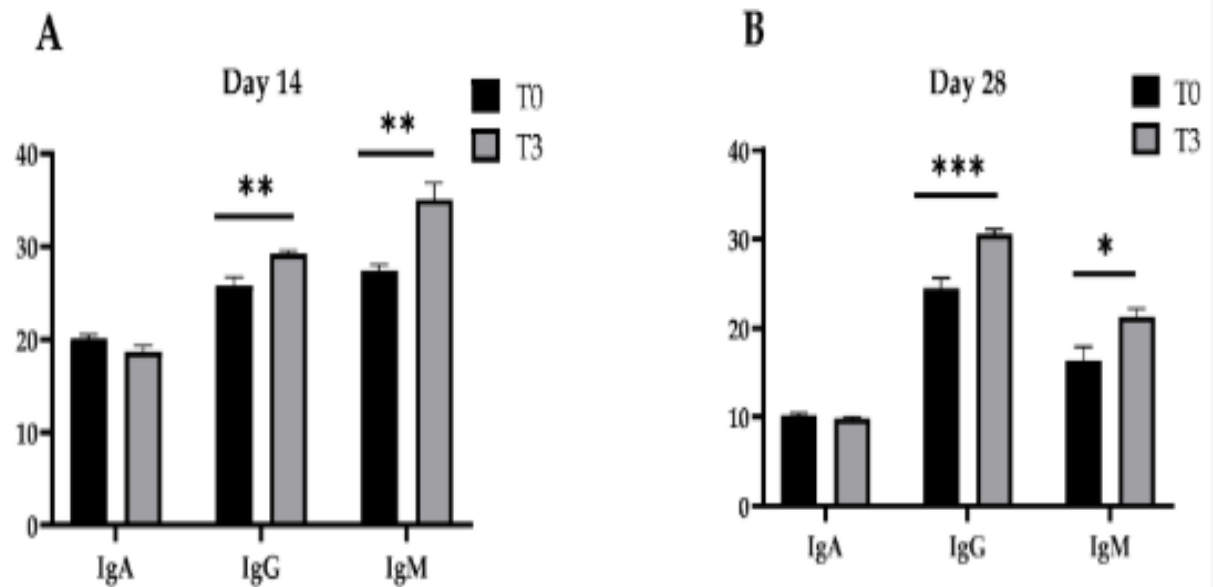


Figure 2. Effect of dietary supplementation with AOLP on plasma immune concentration (mg/mL) of weaned piglets on day 14 (A) and day 28 (B). The results are presented as mean $\pm$ SEM; $n = 6$. * $p < 0.05$; ** $p < 0.01$; and *** $p < 0.001$ indicate difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.



Antioxidant and immunomodulatory properties

Results

Figure 3. Effects of dietary supplementation with AOLP on morphology of duodenum (A), jejunum (B), and ileum (C) of weaned piglets. VH, villus height; CD, crypt depth; V-C, villus height-to-crypt depth ratio. Results are presented as mean $\pm$ SEM; $n = 6$. * $p < 0.05$ and ** $p < 0.01$ indicate difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.

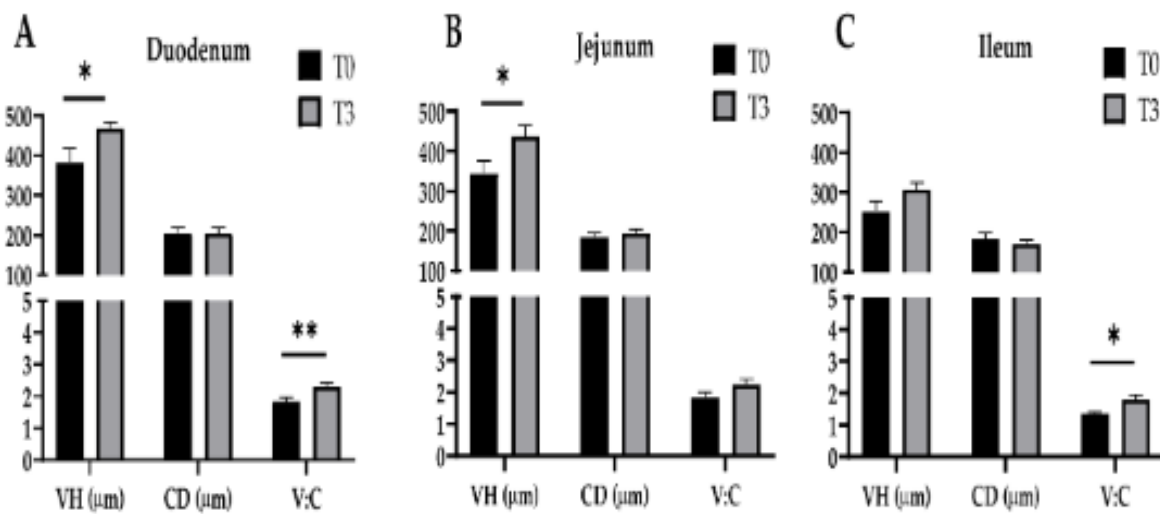
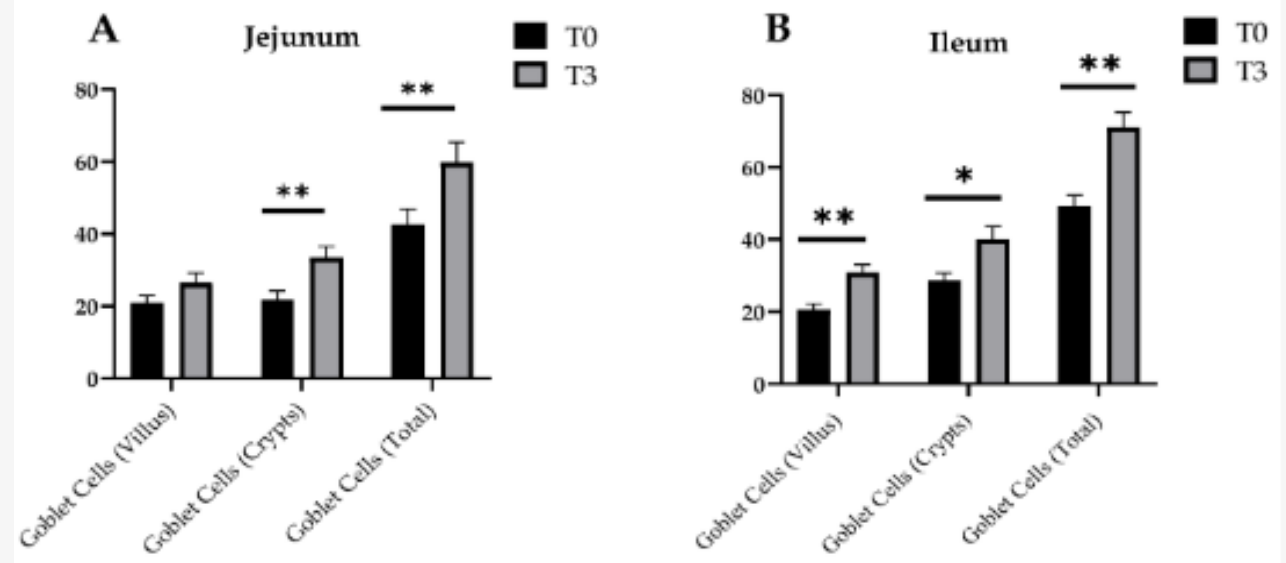


Figure 4. Effects of dietary supplementation with AOLP on number of goblet cells of jejunum (A) and ileum (B) of weaned piglets. Results are presented as mean $\pm$ SEM; $n = 6$. ** $p < 0.01$ and * $p < 0.05$ indicate difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.



Better gut health

Results

Figure 5. Effects of dietary supplementation with AOLP on intestinal Mucin2 area. Results are presented as mean $\pm$ SEM; $n = 6$. * $p < 0.05$ indicates difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.

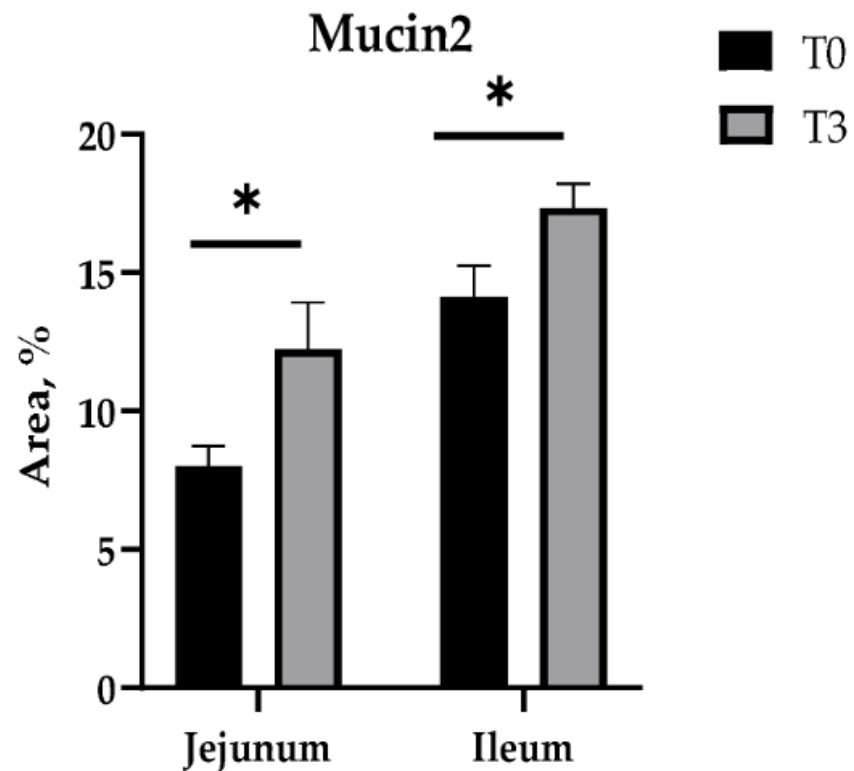
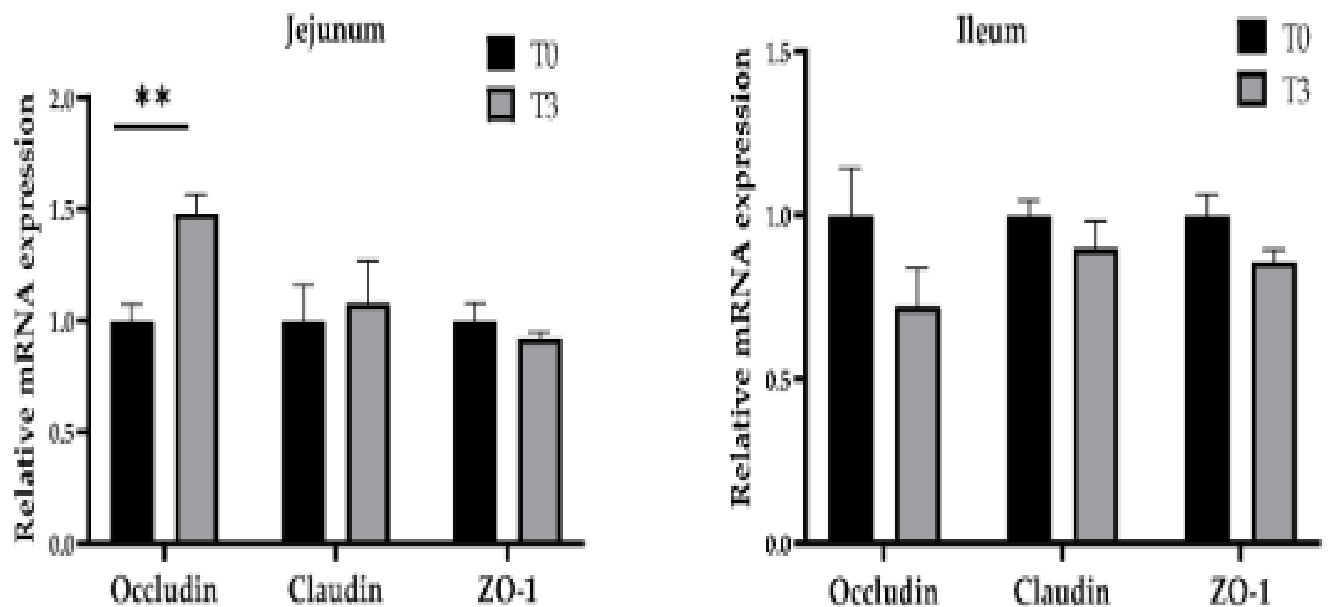


Figure 6. Effects of dietary supplementation with AOLP on mucosal relative gene expression in jejunum and ileum. Results are presented as mean $\pm$ SEM; $n = 6$. ** $p < 0.01$ indicates difference between treatments. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.



Intestinal permeability

Results

Figure 7. Effect of dietary supplementation with AOLP on fecal microbiota of weaned piglets (n = 6). (A) Venn diagram of genera in fecal samples. (B) Non-metric multidimensional scaling on genus level. (C) Principal coordinate analysis. (D) Wilcoxon rank-sum test bar plot on genus level and (E) amplicon sequence variants (ASV) level. T0 = control group; T3 = group supplemented with 15 g/kg AOLP.

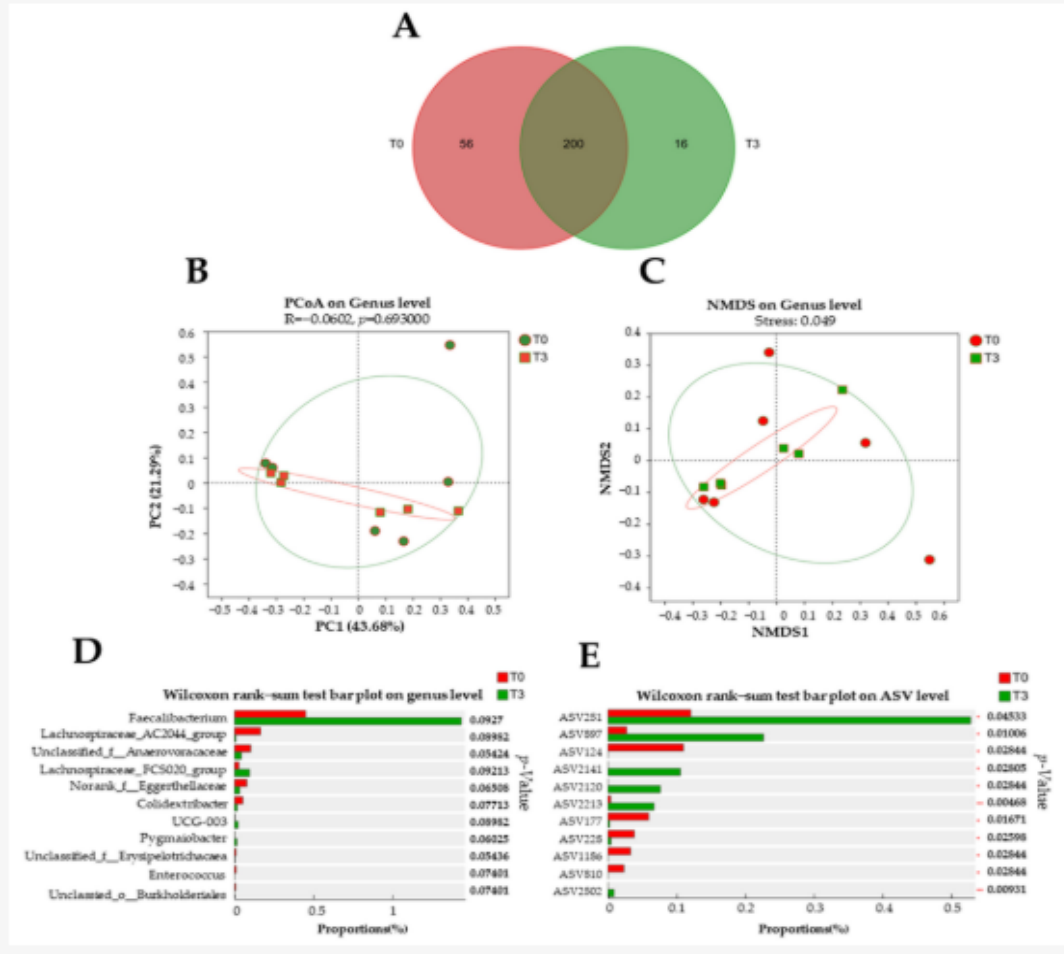
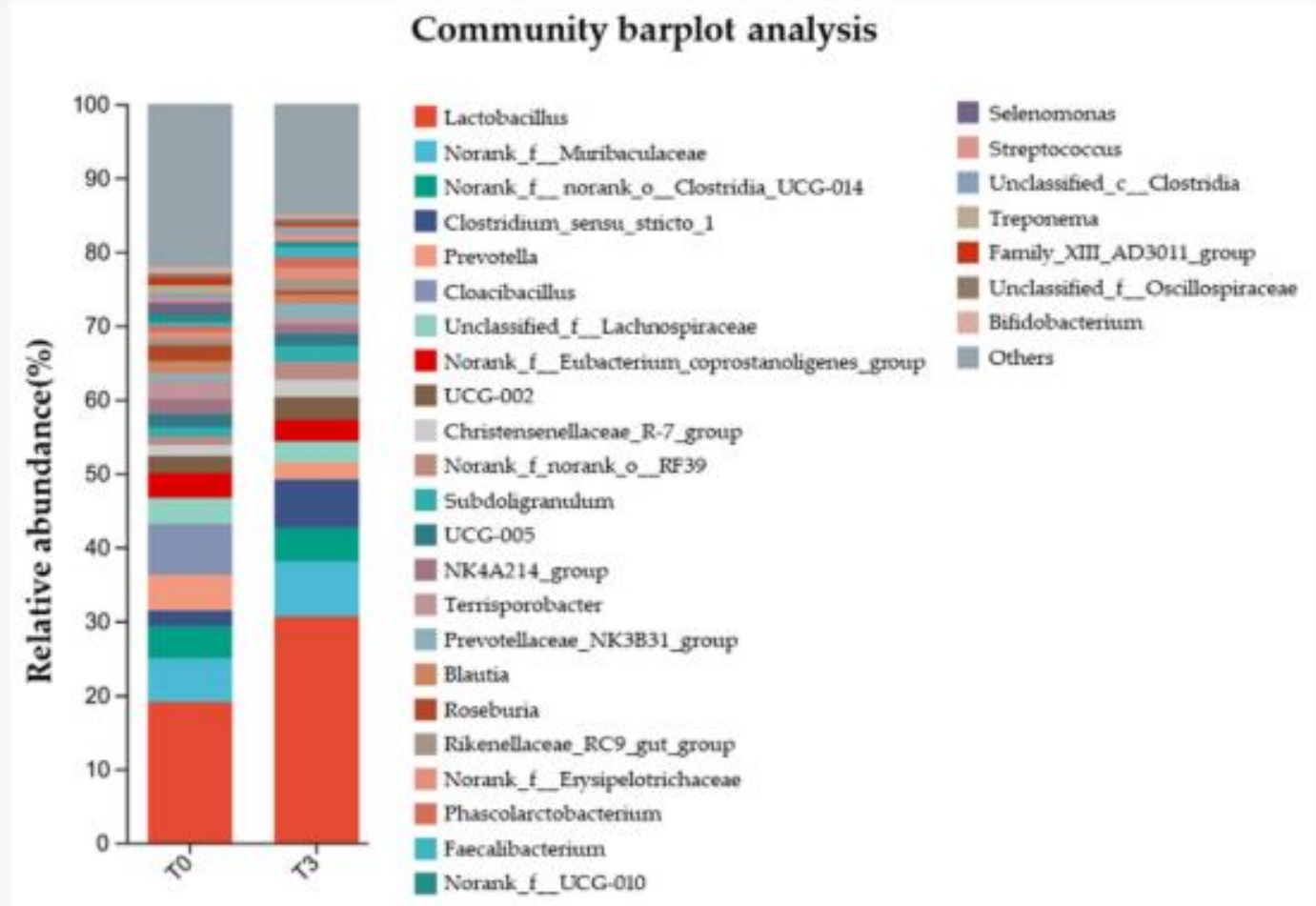


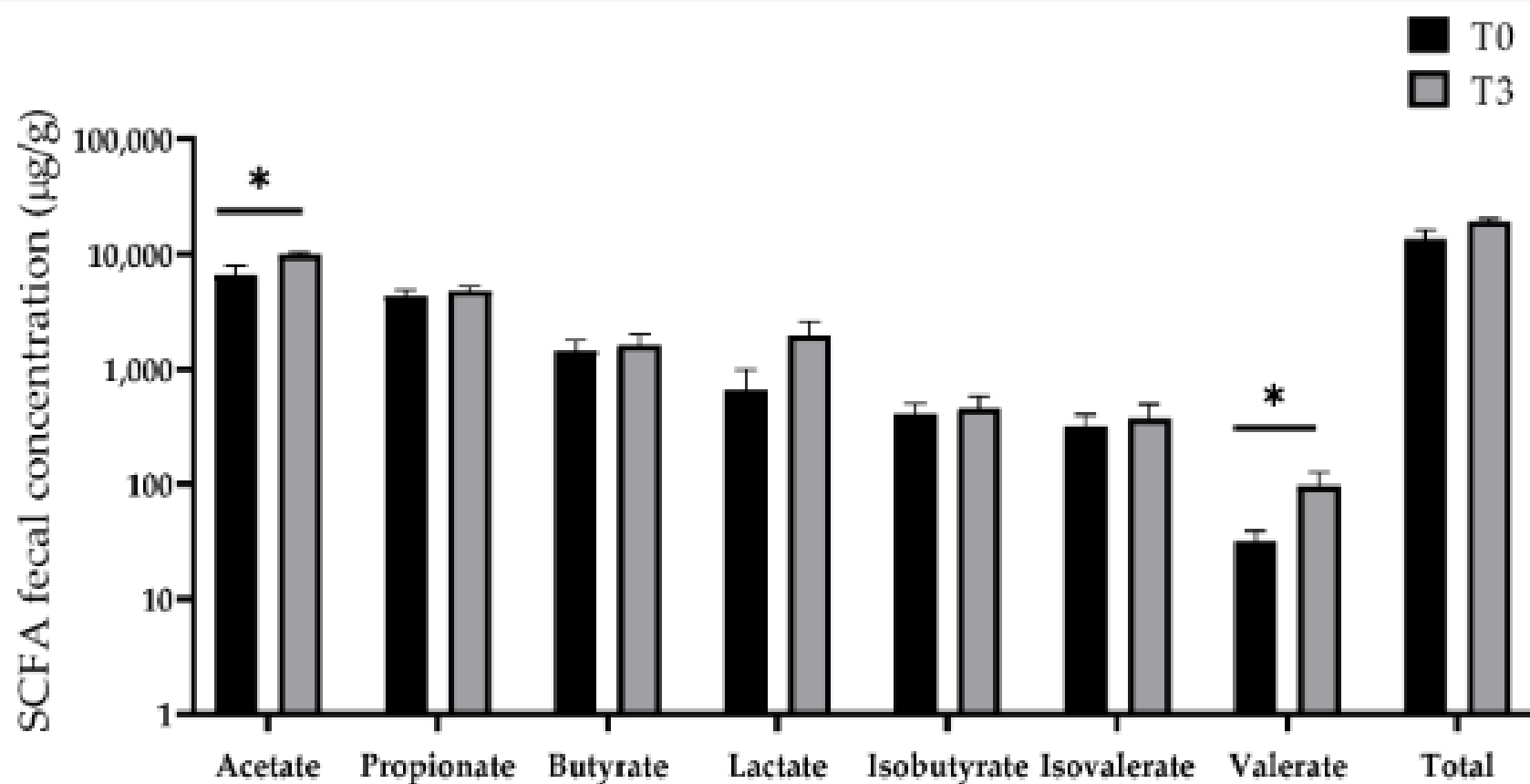
Figure 8. Effect of dietary supplementation with AOLP on relative abundance of gut microbiota at genus level.



Competitive exclusion

Results

Figure 9. Effect of dietary supplementation with AOLP on SCFA production of weaned piglets. Results are presented as mean $\pm$ SEM; $n = 6$. * $p < 0.05$ indicates difference between treatments. SCFA = short-chain fatty acid.



Conclusions

Dietary supplementation with AOLP in weaned piglets decreased the diarrhea incidence, without changing the productivity of the pigs. Dietary supplementation with 1.5% of AOLP decreased the plasma concentration of MDA and increased the concentrations of IgG and IgM, as well as improving the intestinal integrity and the production of acetate and valerate in the large intestines of the pigs.

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