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Acidifiers and prebiotics in the diets of nursery-stage piglets as alternatives to antibiotic growth promoters

Associação de acidificantes e prebióticos para leitões em fase de creche como alternativa aos antibióticos promotores de crescimento

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Highlights

Organic acids and prebiotics promote feed efficiency in piglets.
The use of organic acids and prebiotics reduces the incidence of diarrhea in piglets.
Organic acids and prebiotics decrease the abundance of *E. coli* in the intestine.

Abstract

The objective of this study was to evaluate the performance and intestinal health of nursery-phase piglets (22 to 64 days of age) fed a diet supplemented with organic acids and prebiotics or a diet supplemented with colistin. One hundred fifty weaned piglets 22 days old and with an initial weight of 5.56 kg were allotted to pens based on BW in a completely randomized block design to one of three treatment diets: T1, negative control; T2, colistin (10 mg/kg); and T3, mannan oligosaccharides + beta-glucans + ammonium formate, formic acid, ammonium propionate and acetic acid (1 kg/ton). The daily feed intake, daily weight gain, feed conversion, diarrhea score, cecal *Lactobacillus, E. coli* and fecal coliform content, and proinflammatory and anti-inflammatory cytokine concentrations were evaluated. The performance results considering the whole nursery phase showed a better ($P < 0.05$) feed conversion for T3 (1.593) and T2 (1.602) compared with T1 (1.679). The diarrhea score of T2 was lower than that of T1 ($P < 0.05$) and similar to that of T3 ($P \geq 0.05$). The piglets that received T3 presented a greater concentration of proinflammatory cytokines at 36 days of age than T1, similar to T2 ($p < 0.05$), with the exception of TNFa, where T3 was superior to all groups,

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whereas at 57 days of age the piglets that received T2 presented a greater concentration of inflammatory cytokines. The piglets fed diets supplemented with a combination of mannan oligosaccharides, beta-glucans and acidifiers, representing an alternative to growth-promoting antibiotics, performed similarly to those fed colistin-supplemented diets.
Key words: Antimicrobial replacement. Colistin. Feed additives. Intestinal health. Performance.

Resumo

O objetivo deste trabalho foi avaliar o desempenho e a saúde intestinal de leitões em fase de creche (22 aos 64 dias de idade) submetidos a dietas suplementadas com ácidos orgânicos e prebióticos frente a uma dieta com colistina. Cento e cinquenta leitões desmamados com 22 dias de idade e 5,56 kg de peso inicial foram alojados em baias de acordo com o peso inicial em um desenho em blocos completamente casualizados, sendo submetidos a três tratamentos: T1 - Controle negativo; T2 - Controle positivo (Colistina - 10 mg/kg de ração); e T3 - Mananooligosacarídeos + Betaglucanos + Formiato de amônio, Ácido fórmico, Propionato de amônio e ácido acético (1 kg/ton). Foram avaliados consumo diário de ração, ganho de peso diário, conversão alimentar, escore de diarreias e a quantificação de Lactobacillus, *E. Coli* e Coliformes do conteúdo cecal, e citocinas pró e anti-inflamatórias. O desempenho mostrou uma conversão alimentar melhor (P<0,05) para T3 (1,593) comparado com T1, sendo similar ao grupo T2, considerando toda a fase de creche. O escore de diarreia foi inferior para T3 em relação ao T1 (P<0,05) e semelhante a T2 (P≥0,05). Animais submetidos ao blend de ácidos mais prebióticos (T3) apresentaram maior quantidade de citocinas pró-inflamatórias aos 36 dias de vida em comparação ao grupo T1 (p<0.05), já aos 57 dias de vida o grupo T2 expressou maior quantidade de citocinas inflamatórias. Dietas de leitões na fase de creche suplementadas com uma combinação de Mananooligosacarídeos, betaglucanos, e acidificantes propiciam desempenho semelhante a dietas suplementadas com colistina, representando uma alternativa aos antibióticos promotores de crescimento.
Palavras-chave: Aditivos alimentares. Colistina. Desempenho. Saúde intestinal. Substituição antimicrobiana.

Introduction

In commercial pig farming, weaning is still a challenge for piglets due to the combined effects of various stressors (Wensley et al., 2021), which often have negative effects on digestive function, leading to impaired nutrient absorption, increased diarrhea and worse performance in the first weeks following weaning (Huting et al., 2021; Jayaraman & Nyachoti, 2017; Papadopoulos et al., 2017; Zheng et al., 2021).

When piglets are weaned, even when initiated at a minimum age of 28 days, as stipulated by the European Directive 2008/120/EC (2009), their gastrointestinal tracts are still physiologically and enzymatically immature (Suiryanrayna & Ramana, 2015), and the piglets pass through an immune gap (Jayaraman & Nyachoti, 2017). Because of digestive enzyme insufficiency in this phase, there is an increase in the osmolarity of digestive tract content, resulting in osmotic diarrhea. Additionally, nonabsorbed nutrients serve as substrates for *Escherichia coli,* favoring their colonization and digestive disorders (Corassa et al., 2006). Additionally, limited hydrochloric acid production in newly weaned piglets, which is aggravated by the supply of prestarter feed with high protein content, worsens gastric proteolysis, with consequent intestinal disorders (Roth & Kirchgessner, 1998).

Weaning also affects the innate and adaptive immune development of piglets, affecting the expression of proinflammatory cytokines (Pié et al., 2004; Rymut et al., 2021), including tumor necrosis factor (TNF), interferons (IFNs) and interleukins (ILs), which induce changes in the structure of the intestinal epithelium, increasing its permeability (Nordgreen et al., 2020; Sido et al., 2017).

For decades, the use of antibiotics at this stage of life represented an effective resource for minimizing these consequences; however, the restriction or banning of these additives, arising from the risks of transmission of bacterial selection genes to production animals and to humans, the possible presence of residues in the meat (Vondruskova et al., 2010) and the possibility of the potentiation of diseases (Khanna et al., 2008; Mevius et al., 2009), has become a further challenge for postweaning; in addition, the exclusion of high levels of zinc oxide has exacerbated this problem (Lekagul et al., 2019; Li, 2017).

In this context, a series of additives, called alternatives to antibiotics, such as prebiotics and organic acids, has gained prominence; the actions of these additives have greater amplitude because they promote a better intestinal health status through a reduction in pH in the gastrointestinal tract, the stimulation of enzyme secretion, an increase in digestibility, indirect competitive exclusion, nutrients via nonpathogenic bacteria, direct and indirect antimicrobial effects, and energy input, among other effects (Freitas et al., 2006; Suiryanrayna & Ramana, 2015; Tsiloyiannis et al., 2001), culminating in improved performance.

Regarding acids as additives, most dietary acids are used in the condition of blends, and their responses depend on the level of inclusion, dietary components and nutritional characteristics (Dahmer & Jones, 2021). In parallel, prebiotics derived from the cell wall of the yeast *Saccharomyces cerevisiae,* such as beta-glucans and mannan oligosaccharides, are important representatives of this class of additives. Beta-glucans play an important role in modulating the immune system, associated with anti-inflammatory action (Brown & Gordon, 2003), while mannan oligosaccharides reduce the binding sites of pathogenic bacteria in the intestine, favoring beneficial flora and reducing excessive renewal of the intestinal mucosa, actions that increase animal performance (Tester & Al-Ghazzewi, 2017).

However, prebiotics and organic acids are not commonly used and are known to have different degrees of effectiveness because of the characteristics of the active ingredients that compose these products, such as the types of acids and prebiotics present, the concentrations used, whether the products are blends, aspects related to health challenges present on farms, and the complexity and nutritional profile of the feeds used, among other factors (Suiryanrayna &

Ramana, 2015). Thus, the aim of this study was to evaluate the performance, diarrhea control and intestinal flora modulation in nursery-phase piglets fed a diet with a mixture of prebiotics and organic acids or a diet with growth-promoting antibiotics.

Materials and Methods

All experimental procedures were conducted in accordance with animal welfare principles and submitted to the Ethics Committee on Animal Experimentation (CEUA) of the AKEI Animal Research Center, protocol No. 005.19.

A total of 150 piglets (75 castrated males and 75 females; PIC® genetics, Camborough x AG 337) with a mean age of 22 days and live weight of 5.568 ± 0.781 kg were used. The animals were housed in pens (2.55 m2) equipped with a fully slatted floor, pendulum-type drinker (with adjustable height) and linear feeders with 6 mouths. The environment was thermally controlled using 250 W infrared lamps installed in the center of the bay and side curtains.

This study was conducted with a randomized block design (initial weight of the animals and sex), with 3 treatments and 10 replicates per treatment, with a pen with 5 animals of the same sex being the experimental unit. The experimental treatments were as follows: T1, negative control; T2, positive control (colistin, 10 mg/kg); and T3, blend of organic acids and prebiotics [ammonium formate, formic acid, ammonium propionate and acetic acid + mannan oligosaccharides + beta-glucans, 1 kg/ton (Uniwall MOS 50®, Vetanco, Buenos Aires, Argentina)].

The animals received water and feed *ad libitum* throughout the experimental period (42 days) under a commercial nutritional program comprising 4 phases (Table 1). Weight management assessments and feed intake calculations, after deducting leftovers and losses, were performed at weekly intervals; the piglets were weighed individually. To evaluate performance, daily feed intake (DFI), daily weight gain (DWG) and feed conversion (FC) were calculated, and values were expressed weekly, phase by phase and throughout the experimental period.

Table 1
Composition of and nutritional values for the feeds used in the experimental procedures pre-initial phases I and II and initial phases I and II

Ingredient	Phases			
	Pre-initial I	Pre-initial II	Initial I	Initial II
Corn	44.142	42.685	51.412	60.630
Soybean meal	13.161	22.048	26.352	30.838
Precooked corn	10.000	10.000	5.000	0.000
Whey powder	14.000	12.000	7.000	0.000
Milk powder	10.000	5.000	2.500	0.000

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Plasma	5.000	4.000	2.500	0.000
Soybean oil	0.208	1.075	1.519	4.199
Dicalcium phosphate	1.110	1.073	1.452	1.833
Limestone	0.280	0.506	0.547	0.765
L-lysine	0.543	0.316	0.365	0.397
L-threonine	0.256	0.121	0.150	0.157
DL-methionine	0.380	0.214	0.185	0.156
L-valine	0.039	0.000	0.000	0.033
L-tryptophan	0.051	0.012	0.012	0.024
Adsorptive	0.150	0.150	0.150	0.150
Common salt	0.032	0.132	0.233	0.568
Banox	0.010	0.010	0.010	0.010
Premix vitamin 1	0.150	0.150	0.150	0.150
Premix mineral 2	0.100	0.100	0.100	0.100
Total	100.00	100.00	100.00	100.00
Nutrients				
Protein, %	18.500	20.045	20.000	19.450
Fat, %	4.837	4.383	4.461	6.640
Crude fiber, %	1.801	2.355	2.682	3.053
Calcium, %	0.64	0.697	0.750	0.872
Available phosphorus, %	0.440	0.401	0.420	0.431
Metabolizable energy, kcal/kg	3.500	3.400	3.350	3.350
Lysine dig, %	1.440	1.334	1.300	1.206
Methionine + dig cysteine, %	0.927	0.814	0.770	0.687
Threonine dig, %	0.940	0.855	0.840	0.784
Tryptophan dig, %	0.250	0.240	0.230	0.229
Valine dig, %	0.857	0.892	0.813	0.832
Sodium, %	0.320	0.300	0.250	0.250
Chlorine, %	0.380	0.346	0.337	0.366

[1] levels per kg of premix vitamin product: vitamin A (min), 6,000 IU; vitamin D3 (min), 1,500 IU; vitamin E (min), 15,000 mg; vitamin K3 (min), 1,500 mg; vitamin B1 (min), 1.350 mg; vitamin B2, 4,000 mg; vitamin B6, 2,000 mg; vitamin B12 (min), 20 mg; niacin (min), 20,000 mg; pantothenic acid (min), 9.350 mg; folic acid (min), 600 mg; biotin (min), 80 mg; selenium (min), 300 mg. 2 levels per kg of Premix mineral product: iron (min), 100 mg; copper (min), 10 mg; manganese (min), 40 g; cobalt (min), 1,000 mg; zinc (min), 100 mg; iodine (min), 1,500 mg.

The diarrhea score, determined using the method described by Liu et al. (2019), was classified as follows: 0, feces with normal consistency; 1, soft feces; 2, pasty feces; and 3, aqueous feces. The diarrhea index was calculated using the following formula:

- Diarrhea index = number of days with diarrhea/total number of days of the test

On the 24th day of housing (46 days of age), 1 animal from each stall was randomly selected, euthanized in accordance with animal welfare stipulations, and desensitized using a Petrovina IS 200 with 2 electrodes (250 V and 1.3 A for 3 seconds). Subsequently, 2 g of cecal content was collected, placed in an Eppendorf tube, labeled and stored under refrigeration for subsequent quantification of Lactobacillus and Escherichia coli and total coliforms in accordance with Instruction No 62 (Instrução Normativa, 2003).

To determine the concentrations of interleukin (IL) 1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, interferon alpha (IFN-α) and gamma (IFN-ɣ) and tumor necrosis factor alpha (TNFα), blood samples were collected from 30 piglets, 1 from each replicate, half males and half females, randomly selected at 2 time points (36 and 57 days of age). After collection, the samples were processed and analyzed using a Luminex xMAP following the protocol provided with a commercial kit (Invitrogen™, EPX090-60829-901).

All data were statistically analyzed using R Statistical Program, version 3.3.0 (2016-03-05). Prior to statistical analyses, the outlier results were removed through the box and whisker package, and normality of the data was tested by means of the Shapiro-Wilk test. Nonnormal data were subjected to a nonparametric Kruskal-Wallis test with Dunn's post hoc test. The other results were subjected to ANOVA, and the means were compared by Tukey's test; differences were declared significant at $P<0.05$ and a trend at $P<0.10$.

Results and Discussion

Performance

There was no difference ($P \geq 0.05$) between the treatments for DFI and live weight at the end of the phases for any of the periods evaluated. However, differences ($P \leq 0.05$) were verified for the daily weight gain (DWG) and feed conversion (FC) in initial phase II between the groups, in which T1 showed the worst result (2.261 versus 1.721 and 1.739 for T1, T2 and T3, respectively). Differences were also observed in the total period of the evaluation, between which T2 and T3 presented better FC than T1 (4.80 and 5.39%, respectively) (Table 2).

The absence of effects on DFI, particularly for the group that received the blend of prebiotics and acids, is supported by the results obtained by Corassa et al. (2006). This finding contrasts with the consumption-promoting effect of the acids because it gives a sweet taste to the food, improving the feed consumption (Lei et al., 2017; Luise et al., 2017).

Regarding the DWG, the best results were observed for the treatments that received the additives during the initial phase II (0.589 and 0.540 versus 0.463 g for T2, T3 and T1, respectively), but these gains were not sufficient to improve the DWG considering the full evaluation period. Our findings were compared with those of Vieira et al. (2017), who worked with a mixture of acetic, propionic and formic organic acids (50%) associated with mannan oligosaccharide (50%) for piglets and only found a better DWG during the third and fourth weeks after weaning for this treatment compared with a control diet. Our findings were similar to those observed by Luna et al. (2015), who evaluated the performance of piglets during the nursery phase and found no advantages for piglets fed diets with mannan oligosaccharides + beta-glucans compared with piglets that received colistin. Other studies in which prebiotics replaced growth-promoting antibiotics (tiamulin) confirm our results, such as Park et al. (2018), who used diets with fructooligosaccharides + beta-glucans at different doses and did not observe differences between treatments.

Table 2
Mean performance values for piglets that received experimental treatments (values expressed in kilograms)

Phase	Treatments			CV (%)	P value
Pre-initial 1	T1	T2	T3		
Initial weight	5.567	5.570	5.569	2.80	0.999
DFI	0.227	0.233	0.231	9.12	0.823
DWG	0.167	0.172	0.168	11.89	0.523
FC	1.422	1.368	1.388	11.79	0.758
Weight	8.132	8.302	8.160	3.66	0.411
Pre-initial 2					
DFI	0.507	0.505	0.498	10.32	0.929
DWG	0.319	0.309	0.311	11.07	0.793
FC	1.590	1.637	1.607	5.64	0.523
Weight	10.697	10.693	10.675	4.68	0.994
Initial 1					
DFI	0.784	0.792	0.788	9.41	0.966
DWG	0.471	0.464	0.465	7.74	0.891
FC	1.665	1.708	1.694	7.18	0.715
Weight	17.141	16.795	16.915	4.48	0.535
Initial 2					
DFI	1.028	1.008	0.932	11.17	0.143
DWG	0.463b	0.589a	0.540a	12.61	0.001
FC	2.261b	1.721a	1.739a	16.95	0.001
Final weight	20.387	20.883	20.693	4.42	0.481
Total					
DFI	0.599	0.600	0.584	7.38	0.671
DWG	0.356	0.375	0.366	6.01	0.197
FC	1.679b	1.602a	1.593a	5.42	0.070

[a-b] means followed by different letters in rows indicate differences by Tukey's test ($P < 0.05$). T1, negative control; T2, positive control; T3, acids + prebiotics. CV, coefficient of variation. DFI, daily feed intake, DWG, daily weight gain and FC, feed conversion.

The results for FC obtained for initial phase II and for the entire experimental period were similar to the results observed by Freitas et al. (2006), who reported advantages with diets with organic acids. The improvement in FC with the use of acidifiers in the diet corroborates the results obtained by Braz et al. (2011), which can be attributed to the better use of dietary protein (Freitas et al., 2006) and to the classic antimicrobial action that organic acids exert (Roth & Kirchgessner, 1998).

The positive effects of the combination of acid and prebiotic blends indicate the effectiveness of its actions on intestinal health, with a consequent effect on feed efficiency (Freitas et al., 2006). Although there are still some variations in zootechnical results when growth-promoting antibiotics are replaced by these additives, a large number of studies report these benefits (Lei et al., 2017; Luise et al., 2017; Papadopoulos et al., 2017).

Intestinal health and inflammatory response

The combined use of acids and prebiotics (T3) for the control of diarrhea was effective (Table 3), especially for piglets with more severe diarrhea, i.e., score 3 (liquid/aqueous); the animals that received the acids with prebiotics showed an intermediate result (P > 0.05) compared with those for the negative control (T1) and colistin (T2) groups. The results obtained are similar to those reported by Tsiloyiannis et al. (2001) and Freitas et al. (2006), who observed lower diarrhea scores for weaned piglets that received diets with acidifiers than those that received diets without additives (negative control).

Table 3
Occurrence of diarrhea, score 2, score 3 and total score (2 + 3) in piglets that received experimental treatments (values expressed in number)

Scores	Treatments		
	T1	T2	T3
2	0	0	0
3	21b	09a	14ab
Diarrhea index	0.42	0.18	0.28

[a-b] means followed by different letters in rows indicate differences by the chi-square test (P < 0.05). T1, negative control; T2, positive control; T3, acids + prebiotics.

The control of diarrhea by acids and prebiotics involves direct and indirect actions, including the preservation of the intestinal epithelium by reducing the concentration of pathogenic bacteria in the lumen of the intestine. Acids, through the dissociation of their molecules in the cytoplasm of microorganisms, play a role in irreversibly reducing the pH and denaturing the DNA of pathogens (Braz et al., 2011). In this sense, this trend toward reducing diarrhea is consistent with the results of other studies (Braz et al., 2011; Freitas et al., 2006; Tsiloyiannis et al., 2001). On the other hand, prebiotics have direct action that stimulates the immune response (Naqid et al., 2015; Wu et al., 2017) and limits the nutritional supply of pathogenic bacteria (Hutkins et al., 2016). These actions may support the better results obtained for piglets fed diets containing the combination of acids and prebiotics.

Regarding the cecal bacteria count, there was no difference among treatments for the genus Lactobacillus (Table 4). The results are similar to those obtained by Callegari et al. (2016), who worked with different blends of acids (encapsulated or not) and/or acids in combination with essential oils and a control diet (free of these additives) and did not observe differences among groups for this genus of bacteria.

Table 4
***Lactobacillus, E. coli* and total coliform count in the feces of piglets after the experimental treatments**

Count	Treatments			CV (%)	P value
	T1	T2	T3		
Lactobacillus (log CFU/mL)	8.022	8.054	7.811	4.81	0.400
E. coli (log CFU/g)	6.982a	8.567b	6.748a	18.99	0.003
Total coliforms (log CFU/g)	6.921a	8.584b	6.844a	18.67	0.005

T1, negative control; T2, positive control; T3, acids + prebiotics. CV, coefficient of variation.

In the group treated with acids and prebiotics (T3), there was a reduction in E. coli and total coliforms compared with that in the group treated with colistin (T2). Acids and prebiotics favor the production of volatile fatty acids in the cecum and colon, inhibiting the development of pathogens, such as E. coli and fecal coliforms, which may explain the situation observed with the use of colistin; similar results were observed in other studies that compared these additives against growth-promoting antibiotics (Callegari et al., 2016; Suiryanrayna & Ramana, 2015; Wu et al., 2017). Additionally, this result may suggest the occurrence of dysbiosis caused by the antibiotic at the dose used. According to Braz et al. (2011), a growth-promoting antibiotic can lead to an imbalance in intestinal microbiota with consequent morphological damage in the intestinal epithelium, in addition to all of the resulting negative repercussions.

However, the results for the control group (T1) were the same as those for the T3 group, a finding that may be due to the complexity of the composition of the feeds used, as they contained several highly digestible ingredients with high concentrations of dairy products. Dairy products (whey powder and whole-milk powder), as ingredients in piglet feed, are important substrates that, when fermented in the gastrointestinal tract, are converted into lactic acid, which in turn initiates acidifying action with all of the expected benefits (Jayaraman & Nyachoti, 2017); these benefits include, as observed, the modulation of the Lactobacillus population.

Regarding the quantification of cytokines and blood markers that are indicative of immune changes, including states of intestinal inflammation (Nordgreen et al., 2020; Yu et al., 2019), for the first collection, performed at 36 days of age (Table 5), the animals in the T3 group presented a higher IL-1β level than did the other treatment groups ($p < 0.05$). For the second collection (performed at 57 days of age), the levels of this interleukin were significantly higher in the T2 group than in the other groups (T1 and T3; $p < 0.05$). For IL-4, for the first collection, the animals in the T3 group had higher levels than those in the T1 group, with T2 having intermediate levels between those for T1 and T3. For the second collection, the animals in the T2 group had IL-4 levels higher than those in the T1 group ($P < 0.05$) and similar to those in the T3 group ($P \geq 0.05$).

For IL-6, there was a trend ($p < 0.10$) in the increase in cytokines in the T3 group compared with those in the T1 group at both collection timepoints. Similarly, the T3 group showed an increasing trend in IFN-α for the first collection; for the second collection, the T2 group had the highest levels, with those for the T3 group being intermediate between those for the T2 and T1 groups ($p < 0.10$). For TNF-α, the levels in the T3 group were higher than those in the T1 and T2 groups for the first collection, with no difference among treatments for the second collection. IL-8, IL-10, IL-12p40 and IFN-γ were not different among the groups for the 2 periods evaluated ($p > 0.05$).

The activation of some inflammatory cytokines, such as IL1-β, TNF-α and IL-6, is strongly associated with postweaning in piglets (Novais et al., 2021; Rymut et al., 2021); increased expression may last for days (Moeser et al., 2017; Novais et al., 2021), confirming our findings, particularly regarding the results for the first collection (Table 5).

As mentioned, many cytokines have adverse effects on the integrity of the intestinal mucosa and its epithelial function (Nordgreen et al., 2020; Pié et al., 2004). In piglets, an increase in intestinal permeability due to inflammation was observed 24 h postweaning, followed by a gradual decline in this state during the first 2 weeks after weaning (Moeser et al., 2017). This effect was also identified in this study; based on the quantification of cytokines, the inflammatory state decreased, given the decline in IL1-β, IL-4, IL-6 and TNF-α levels in the group fed prebiotics and organic acids between the first (36 days) and second (57 days) collections.

Table 5
Concentrations (pg/mL) of interleukins (IL) 1β, IL-4, IL-6, IL-8, IL-10, IL-12p40, interferon alpha (IFN-α) and gamma (IFN- γ) and tumor necrosis factor alpha (TNFα) at 36 and 57 days of age

Count	Treatments			CV (%)	P value
	T1	T2	T3		
First collection (36 days)					
IL-1 β (pg/mL)	1.758b	1.758b	270.767a	212.52	0.0054
IL4 (pg/mL)	0.832b	1.039ab	1.506a	43.10	0.0037
IL6 (pg/mL)	3.868b	7.002ab	13.188a	57.66	0.1078
IL8 (pg/mL)	59.026	39.882	43.864	46.96	0.1369
IL10 (pg/mL)	2.771	2.771	2.771	0.00	1.0000
IL 12p40 (pg/mL)	286.303	659.387	512.927	59.63	0.5764
IFN-α (pg/mL)	0.774b	0.925ab	1.428a	58.96	0.0855
IFN-γ (pg/mL)	7.508	4.383	3.741	53.07	0.3794
TNFα (pg/mL)	2.759b	2.759b	231.267a	199.18	0.0020
Second collection (57 days)					
IL-1 β (pg/mL)	1.758b	435.002a	1.758b	209.10	0.0012
IL4 (pg/mL)	0.909b	2.170a	1.010ab	70.27	0.0005
IL6 (pg/mL)	3.682b	8.811a	8.470a	88.56	0.0743
IL8 (pg/mL)	75.952	54.092	48.970	50.11	0.5212
IL10 (pg/mL)	2.771	2.771	2.771	0.00	1.0000
IL 12p40 (pg/mL)	359.509	856.910	687.669	45.64	0.4179
IFN-α (pg/mL)	0.825b	2.204a	1.264ab	65.37	0.0534
IFN-γ (pg/mL)	4.594	5.090	6.484	48.33	0.8899
TNFα (pg/mL)	2.759	458.849	181.599	279.10	0.2441

[a, b] means followed by different letters in rows indicate differences by Tukey's test ($P < 0.05$). T1, negative control; T2, positive control; T3, acids + prebiotics. CV, coefficient of variation.

Prebiotics, such as oligosaccharides, aid in the production of lactic acid and acetic acid and reduce intestinal pH, favoring digestive enzyme activity, which contributes to the maintenance of intestinal health, with a reduction in intestinal inflammation due to the lower expression of inflammatory cytokines (Zenhom et al., 2011). Inflammatory responses, however, are different among animals given the different factors involved. The microbiological agents to which animals are exposed and noninfectious agents thus determine different responses. A viral infection will generally stimulate the synthesis of interferons (α and β), and a bacterial infection will stimulate the expression of IL1-β, TNF-α and IL-6 (Nordgreen et al., 2020). Pigs, as well as other animals, are prone to several gastrointestinal bacterial diseases, such as colibacillosis, whose agent, a gram-negative bacterium, contains a lipopolysaccharide responsible for activating a cytokine cascade that mediates the inflammatory process (Fairbrother & Nadeau, 2019; Nordgreen et al., 2020).

The best results observed for the T3 group (acids + prebiotics), relative to the lower *E. coli* and total coliform counts at 46 days of life ($p < 0.05$; Table 4), can be explained by the lower expression of inflammatory cytokines ($p < 0.05$) (IL1- β and IL-6) evaluated at 56 days of age (second collection) (Table 5). In contrast, the animals in the T2 group had higher *E. coli* and total coliform counts at 46 days of age; at 56 days of age, the concentration of inflammatory cytokines was higher (Tables 4 and 5).

Prebiotics (such as mannooligosaccharides + beta-glucans) and organic acids (represented in this study by formic acid, acetic acids and ammonium propionate) are active ingredients in the diets of weaned piglets and effective substitutes for PCA, such as colistin (T2). Prebiotics are known to modulate the intestinal microbiota, favoring the production of short-chain fatty acids (Bourgot et al., 2014; Shang et al., 2017), which in turn promote the development of beneficial agents in the gastrointestinal tract (Chen et al., 2017; Lee et al., 2017); through various actions, especially competitive exclusion, these agents lead to a reduction in pathogenic microorganisms, such as *E. coli,* as evidenced at 46 days of life (Table 4). As a consequence of this reduction, a result observed in the animals in the T3 group, there was lower expression of inflammatory cytokines at 56 days of life (Table 5); this may be associated with the smaller number of animals with diarrhea in the period studied (Table 3).

These conditions confirm the benefits of the evaluated blend, which, in initial phase II and in the total period, led to better zootechnical performance for the animals in the T3 group compared with that for the control group (T1), without differences from the results obtained for the group that received colistin (T2).

Conclusions

Diets of nursery-phase piglets supplemented with a combination of acids + mannan oligosaccharides + beta-glucans provide similar performance and better intestinal health compared with diets supplemented with colistin. Based on the results of this study, acidifiers and prebiotics under *blended* conditions represent effective alternatives to the antimicrobial growth promoters currently used in pig farming.

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