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Effects of adding a prebiotic product based of beta-glucans, glucomannans, and mannan-oligosaccharides on performance and health of weanling pigs

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ABSTRACT - The objective of this study was to examine the effects of replacement of an antibiotic (ANT, chlorohydroxyquinoline) in the diet of weaned piglets with a prebiotic composed of β-glucans, glucomannans, and mannan-oligosaccharides on growth performance, diarrhea occurrence (DO), hematological parameters, pH of digestive content, organ weights, intestinal epithelium morphology, and intestinal bacterial counts. A total of 120 piglets (weaned at 21 d old; 6.32±0.10 kg BW) were allocated in a randomized block design, with six pens replicates and four piglets per pen as experimental unit. Treatments consisted of diets with 0, 1,000, 2,000, or 3,000 mg kg^{-1} of prebiotic and a basal diet with 120 mg kg^{-1} ANT. No differences of treatments were observed on performance, DO, pH of digestive content, organ weights, and hematological parameters. Duodenal villus density (VD) increased linearly with the prebiotic levels. In the jejunum, the prebiotic level of 2,000 mg kg^{-1} resulted in a greater VD compared with the ANT. Enterobacteriaceae count in the ileum and total and lactic acid bacteria counts in the cecum responded quadratically to increasing prebiotic levels. The equations estimated the prebiotic levels of 2,125, 1,167, and 1,500 mg kg^{-1} to provide reduced counts of the respective bacteria (ileal enterobacteria: 6.47 log CFU g^{-1}; total cecal bacteria: 6.70 log CFU g^{-1}; and cecal lactic acid: 7.29 log CFU g^{-1}). *Salmonella* spp. was not detected, whereas *Escherichia coli* and *Lactobacillus* spp. gene copies were unaffected by the treatments. Prebiotic levels or ANT do not enhance performance or health of weanling pigs, but the effects observed on VD and microbiota profile might be an indication of the potential benefits of these products in commercial farming conditions.

Keywords: antimicrobial, feed additive, intestinal health, piglet, post-weaning, qPCR

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1. Introduction

The post-weaning period of piglets is marked by anorexia, limited feed digestion and nutrient absorption, and diarrhea, which lead to reduced growth rates (Heo et al., 2013). Adaptive responses of gastrointestinal tissues and organs, microbiota balance, and immune activation have been the main concerns in the nursery phase (Genova et al., 2020). To improve the health status and performance of newly weaned piglets and in view of the risks of bacterial cross-resistance, feed additives have been increasingly developed as an alternative to ANT in animal production (Landy et al., 2020).

Beta-glucans, glucomannans, and mannan-oligosaccharides (MOS) from the cell wall of the yeast *Saccharomyces cerevisiae* are carbohydrates resistant to gastrointestinal enzymatic degradation, so they reach the intestine intact and promote intestinal health. The major mechanisms of action by including β-glucans in piglet diets are stimulus to the immune system and anti-inflammatory effects (Stuyven et al., 2009). Glucomannans and MOS, which are selectively fermented by beneficial bacteria, act by stimulating the immune system and absorbing mycotoxins and pathogenic bacteria through specific binding with fimbrial receptors (Spring et al., 2015; Tester and Al-Ghazzewi, 2017).

Few studies have investigated the synergistic effects of β-glucans, glucomannans, and MOS in the diet of newly weaned piglet. Luna et al. (2015) observed an increase in intestinal villus height in piglets fed β-glucans and MOS, whereas Thi Tuoi et al. (2016) reported improvements in performance, bacterial population balance, diarrhea occurrence (DO), and immune response. Nonetheless, results have been inconclusive regarding the optimum level to be used in the diet, how to obtain the products, and use period.

Our team published the first study evaluating the association of these three prebiotics in the diet of weaned pigs, in over a 35-d experimentation period (Anjos et al., 2019). Here, we hypothesized that prebiotic additives combined at different inclusion levels could be promising for bolstering growth and health in the diet of piglets raised without prophylactic antibiotics. Therefore, aiming to understand the benefits of these prebiotics in the period of greatest stress and challenge to the animals (first 14 d of weanling), the present study proposed to assess the replacement of an ANT (chlorohydroxyquinoline) in the diet of newly weaned piglets with graded levels of a prebiotic additive based on β-glucans, glucomannans, and MOS on growth performance, DO, hematological parameters, pH of digestive content, organ weights, intestinal epithelium morphology, and intestinal bacterial counts.

2. Material and Methods

The study was conducted on an experimental farm located in Marechal Cândido Rondon, Paraná, Brazil (24°31'52" S and 54°01'03" W). Research on animals was conducted according to the institutional committee on animal use (protocol no. 28/2016).

2.1. Animals, housing, experimental design, and dietary treatments

The experiment involved 60 entire male and 60 female piglets (weaned at 21 d-old; 6.32±0.10 kg body weight [BW]), totaling 120 animals (Landrace × Large White, Agroceres♂ and DanBred♀) assigned in a randomized complete block design consisting of two assays in time (two batches of piglets), three blocks of BW within each assay, and five treatments, that is, 15 experimental units per assay with six pens replicates per treatment. Four animals were used in each experimental unit.

The animals were housed in a masonry nursery facility, with tilt-and-turn glass windows and ceiling lined with ceramic tiles at the beginning of the experimental period. Piglets were weighed and distributed in suspended pens (1.54 m^2) containing polyethylene plastic flooring, nipple drinkers, gutter feeders, and heating provided using individual infrared incandescent lamps, where they remained for a period of 14 d.

Diet and water were available *ad libitum* throughout the experimentation period. The control diets (Table 1) were formulated according to the nutritional requirements of weaning piglet as proposed by the NRC (2012), and the feeding program was separated by phases: pre-starter 1 (1 to 7 d) and pre-starter 2 (7 to 14 d).

The treatments were composed of a control diet without feed additive (negative control - 0); control diet supplemented with graded levels (1,000, 2,000, or 3,000 mg kg^{-1}) of a prebiotic additive based on β-glucans, glucomannans, and MOS; and control diet with 120 mg kg^{-1} ANT (positive control; chlorohydroxyquinoline). The prebiotic was a commercial product with the minimum guaranteed levels of 420 g glucomannans, 300 g β-glucans, and 120 g MOS kg^{-1}. All steps of the component extraction and hydrolysis process were according to those described by Anjos et al. (2019).

Table 1 - Centesimal composition and calculated values of diets provided to the animals in the experimental period (as-fed basis)

Item	Pre-starter I (1 to 7 d)	Pre-starter II (7 to 14 d)
Ingredient (%)		
Maize grain, 7.88%	43.46	49.77
Soybean meal, 45.22%	20.24	21.00
Whey powder, 12.3%	11.18	6.27
Micronized soybean, 37%	8.00	6.00
Skim milk powder, 33.4%	6.00	5.00
Sugar	4.00	4.00
Fish meal, 54%	3.00	3.00
Dicalcium phosphate	0.85	0.79
Soybean oil	0.80	1.83
Common salt	0.60	0.58
Limestone	0.60	0.63
L-lysine HCL, 78%	0.44	0.42
DL-methionine, 99.5%	0.28	0.23
L-threonine, 96.8%	0.23	0.19
L-tryptophan, 99%	0.05	0.03
L-valine, 95.5%	0.02	0.01
Vitamin premix[1]	0.15	0.15
Mineral premix[2]	0.10	0.10
Calculated values		
Metabolizable energy (kcal kg^{-1})	3,380	3,400
Crude protein (%)	21.55	20.56
Total calcium (%)	0.85	0.80
Available phosphorus (%)	0.45	0.40
Lactose (%)	11.00	7.00
Digestible lysine (%)	1.45	1.35
Digestible methionine + cysteine (%)	0.79	0.74
Digestible threonine (%)	0.85	0.79
Digestible tryptophan (%)	0.24	0.22
Digestible valine (%)	0.92	0.86

[1] Quantities per kg of diet: folic acid, 2.10 mg; pantothenic acid, 72 mg; biotin, 0,42 mg; ethoxyquin, 0.25 mg; niacin, 180 mg; vitamin A, 36,000 IU; thiamine, 6 mg; vitamin B12, 90 mg; riboflavin, 25.20 mg; vitamin B6, 12 mg; vitamin D3, 7500 IU; vitamin E, 150 IU; vitamin K3, 12 mg.
[2] Quantities per kg of diet: copper sulfate, 9.97 mg; sodium selenite, 1.20 mg; iron sulfate, 96 mg; iodine, 1.20 mg; manganese sulfate, 59.88 mg; zinc oxide, 150 mg.

2.2. Growth performance and diarrhea occurrence

Average daily weight gain (ADWG) was measured by weighing (Rinnert digital scale, model BPW-5000; Braço do Trombudo, SC, Brazil) the piglets individually on days 1 and 14 of the experiment

and dividing the difference by the number of housing days. Average daily feed intake (ADFI) was determined as the difference between the daily amounts of feed supplied and orts. Feed conversion ratio (FCR) was calculated as ADFI:ADWG. The DO was measured as the percentage of days the animals showed clinical signs of diarrhea, which was monitored daily in the morning (08:30 h) before cleaning the pen, according to Cairo et al. (2018).

2.3. Hematological sampling

Blood sampling (≅ 10 mL) was performed on day 13 of experiment via jugular vein using 0.7 × 30 mm gauge needles from one animal per experimental unit. The piglet was selected based on the closest BW to the average BW of its pen (n = 6). The blood samples were transferred to tubes containing ethylenediamine tetraacetic acid (1 mg mL^{-1}), which were labeled, stored in a cool box (4 °C), and sent to the laboratory, where they were subjected to an automated analysis process (Auto Hematology Analyzer BC-2800Vet, Mindray, Shenzhen, China). Circulating cells including both red (erythrocytes, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin concentration, total proteins, and platelets) and body defense cells (leukocytes, segmented neutrophils, eosinophils, lymphocytes, and monocytes) were quantified.

2.4. Organ weights and pH of digestive tract contents

The piglet sampled for blood collection was slaughtered (on day 14 of experimentation after a 6 h-fasting) stunned by electronarcosis and euthanized by exsanguination. The relative weights (stainless steel digital scale, model UL50i; Beijing, China) of the digestive organs (stomach, liver, small intestine and pancreas, cecum, and colon), gall bladder with liver, and spleen were calculated considering the animal's BW at slaughter. The pH of digestive tract contents (stomach, jejunum, ileum, cecum, and colon) was measured by inserting a portable pH meter (HI99163, Hanna Instruments, São Paulo, SP, Brazil), according to Manzanilla et al. (2004).

2.5. Intestinal morphology

For the assessment of the villus height, villus width, crypt depth, and villus height:crypt depth ratio, segments of 3-cm length of duodenum (15-cm from the pyloric junction) and jejunum (150-cm from the ileocecal junction) were sampled, washed with physiological solution (NaCl 0.9%), and fixed in 10% buffered formaldehyde solution. Then, the samples were placed in individual histological cassettes, paraffin-embedded, microtomized (Leica RM2245, Leica Biosystems; São Paulo, SP, Brazil), and stained for mounting the slides (Gao et al., 2000). Fifteen measurements of villi and respective crypts were taken per sample.

For ultrastructural analysis of the same segments (duodenum and jejunum), the collected samples were washed with physiological solution (NaCl 0.9%), fixed in glutaraldehyde, and processed according to Rigueira et al. (2013). The number of villi was counted in distinct fields of each sample. Electron micrographs (n = 5 areas per sample) were obtained for the estimation of villus density (VD; number of villi:μm^2). Then, the observation field was measured, the area determined, and the VD per area calculated (villus:905.216 μm^2).

2.6. Microbial counts

Digestive tract contents from the jejunum (collected by scraping with glass slides at 150-cm from the ileocecal junction), ileum (15-cm from the ileocecal junction), cecum and colon (segment was tied from both ends and the contents homogenized) were collected, stored in identified sterile containers and transported under refrigeration for laboratory analyses. The total counts of aerobic bacteria, Enterobacteriaceae (EMB levine agar, Kasvi), and lactic acid bacteria (MRS agar, Acumedia) were

determined within 24 h by using the standard counting method, in which samples were plated into specific culture media, according to Mookiah et al. (2014).

2.7. Deoxyribonucleic acid extraction

A 0.1-g sample was weighed for DNA extraction using the PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA), following the manufacturer's protocol. Absorbance was measured in the range of 260 to 280 nm, using the NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, Massachusetts, USA), to verify the quantification of the extracted DNA. The DNA samples were diluted to 50 ng μL^{-1} and subjected to a real-time polymerase chain reaction (qPCR) using the TaqMan system (Life Technologies) to determine the number of bacterial gene copies (Table 2).

Table 2 - Primers and probes used in qPCR

qPCR	Target gene	Primers 2 (5'-3') and probes	bp	Reference
Multiplex 1 *Salmonella* spp. and *Escherichia coli*	*phoA*	F: CCGGGTAACGCTCTGGAA R: AGCAGCTGTTCGGTAATCGA P: AAGGCGGAAAAGG (NED)	54	Lopes et al. (2018)
	ssf	F: CGGCGAATTTTTGCGACTAT R: TGGCTTCGCTTTATGTTCGA P: AGGTTACCGTGGAGGC (FAM)	59	Lopes et al. (2018)
Lactobacillus spp.	AY763429	D: GAGGCAGCAGTAGGGAATCTTC R: GGCCAGTTACTACCTCTATCCTTCTTC P: ATGGAGCAACGCCGC (FAM)	126	Delroisse et al. (2008)

2.8. qPCR for quantification of *Salmonella* spp., *Escherichia coli*, and *Lactobacillus* spp.

Standard curves used in qPCR for bacterial quantification were performed according to Lopes et al. (2018) and Delroisse et al. (2008), using serial dilutions (10X) of the target genes from each bacterium, as follows: *ssf Salmonella* (8.64×10^1 to 8.64×10^6 copies, with coefficient of determination (R^2) = 0.998), *phoA E. coli* (7.2×10^1 to 7.2×10^5 copies, with $R^2 = 0.992$), and AY763429 *Lactobacillus* (2.94×10^1 to 2.94×10^6 copies, with $R^2 = 0.998$).

The first multiplex qPCR reaction was performed using the TaqMan system for the detection of *Salmonella* spp. and *E. coli* (Lopes et al., 2018). Amplification was performed in a final volume of 20 µL, containing, in the same reaction, 0.5 µL of specific primers for the amplification of *Salmonella* and *E. coli*, all at the concentration of 5 µM; 0.5 µL of each MGB TaqMan probe specific for *Salmonella* (FAM) and *E. coli* (NED) at a concentration of 5 µM (Table 2); 10.0 µL TaqMan Fast Advanced reagent (Applied Biosystems); and 2.0 µL DNA at 50 ng μL^{-1}. The reaction volume was made up to 20 µL with sterile ultrapure water (free of DNase and RNase).

The second qPCR reaction was performed using the TaqMan system for the detection of *Lactobacillus* spp. (Delroisse et al., 2008). Amplification was carried out in a final volume of 20 µL, containing, in the same reaction, 0.4 µL of specific primers for the amplification of *Lactobacillus* (Table 2), all at the concentration of 5 µM; 0.4 µL MGB TaqMan probe (FAM); 10.0 µL TaqMan Fast Advanced reagent (Applied Biosystems); and 2.0 µL DNA at 50 ng μL^{-1}. The reaction volume was made up to 20 µL with sterile ultrapure water (free of DNase and RNase).

The reactions were amplified in an AB 7500 Fast thermal cycler (Life Technologies, Carlsbad, California, USA), under the following cycle protocol: one cycle at 50 °C for 2 min; one cycle at 95 °C for 20 s; and 45 cycles at 95 °C for 3 s (step 1), 60 °C for 30 s (step 2). The data collection occurred at step 2.

2.9. Statistical procedures

The study was assessed in a combined analysis (Yates and Cochran, 1938; Gomes and Guimarães, 1958). First, we analyzed the assays (batches of piglets) separately to determine whether the variances of the experimental errors in the different assays were homogeneous. This assumption was tested by the F test, with a 1% significance level. Then, the combined analysis was performed using the statistical model:

$$Y_{ijk} = \mu + b_{k(j)} + \alpha_i + \tau_j + \gamma_{ij} + \varepsilon_{ijk},$$

in which Y_{ijk} = observation of treatment i (i = 1, 2, 3, 4, 5) in assay j (j = 1, 2) and in BW block k (k = 1, 2, 3); μ = constant; $b_{k(j)}$ = fixed effect of BW block k in assay j; α_i = fixed effect of treatment i; τ_j = fixed effect of assay j; γ_{ij} = fixed interaction effect between treatment i and assay j; and ε = experimental error.

For the analysis of variance (ANOVA), the residues were tested for normality and homoscedasticity. When ANOVA was significant (P<0.05), two contrast analyses were performed to compare the ANT dietary treatment with each of the others tested by Dunnett's test, and analyzing orthogonal polynomials considering the treatments containing 0, 1,000, 2,000, and 3,000 mg kg^{-1} prebiotic additive. Least significant difference test was applied when the polynomial degree was greater than 2. All analyses were performed using R® software.

3. Results

3.1. Growth performance and diarrhea occurrence

No treatment effect was observed (P>0.05) on growth performance and DO (Table 3).

Table 3 - Body weight (BW), average daily gain (ADG), average daily feed intake (ADFI), feed conversion ratio (FCR), and diarrhea occurrence (DO) in 14-d post-weaning piglets fed dietary antimicrobial growth promoter or graded levels of β-glucans, glucomannans, and mannan-oligosaccharides (MOS)

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
Initial BW (kg)	6.32	6.32	6.32	6.32	6.32	0.088	-
Final BW (kg)	8.16	8.64	8.17	8.11	8.51	0.217	0.25
ADG (g day^{-1})	130	165	134	130	159	0.015	0.21
ADFI (g day^{-1})	225	279	260	253	269	0.021	0.34
FCR	1.75	1.71	1.92	1.96	1.80	0.085	0.19
DO (%)	54.50	52.00	51.50	51.50	56.50	36.723	0.71

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and MOS.

3.2. Hematological analyses

No treatment effects were observed (P>0.05) on the erythrocyte histogram (red blood cells hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total proteins, and platelets) or CBC (white blood cells), segmented neutrophils, eosinophils, lymphocytes, and monocytes (Table 4).

Table 4 - Effects of the diets on hematological parameters of piglets at 14 d of experiment

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
Blood count							
Erythrocytes (millions mm^{-3})	6.20	5.75	5.80	5.80	5.80	0.241	0.62
Hemoglobin (g dL^{-1})	10.50	10.10	10.00	10.15	10.00	0.412	0.88
Hematocrit (%)	35.50	34.00	34.00	35.50	34.50	1.005	0.77
VCM (fL)	57.50	60.00	59.50	60.50	60.00	1.980	0.83
HCM (pg)	17.00	18.00	17.00	17.50	17.50	0.438	0.80
CHCM (%)	29.50	30.00	29.00	29.00	29.00	0.774	0.87
Total proteins (g dL^{-1})	5.55	5.55	5.15	5.40	5.45	0.122	0.28
Platelets (thousands mm^{-3})	622000	670167	597833	613666	793166	82154	0.47
Leukogram							
Leukocytes (cel mm^{-3})	16350	22483	22016	23083	23883	2438	0.25
Segmented neutrophils (%)	55.00	51.00	59.00	59.00	62.00	3.715	0.28
Eosinophils (%)	1.17	0.67	1.34	0.42	1.00	0.489	0.68
Lymphocytes (%)	41.50	44.50	35.50	38.50	34.00	3.734	0.29
Monocytes (%)	2.50	3.65	3.50	2.20	2.75	0.867	0.69

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and MOS.

3.3. Organ weights and pH of digestive tract contents

The treatments did not influence (P>0.05) the pH of the digestive contents in the stomach, jejunum, ileum, cecum, and colon or the relative weights of stomach, small intestine + pancreas, liver + gall bladder, spleen, cecum, and colon (Table 5).

Table 5 - Effects of the diets on relative weights (percentage of body weight) and pH of the digestive tract content of piglets at 14 d of experiment

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
Relative organ weights (%)							
Stomach	1.90	2.40	2.45	2.9	2.45	0.375	0.46
Small intestine + pancreas	7.55	7.60	7.60	9.05	7.75	0.381	0.61
Cecum	0.63	1.04	0.96	0.76	0.99	0.118	0.77
Colon	4.45	3.30	3.45	3.55	2.65	0.394	0.07
Liver + gall bladder	3.20	3.40	3.10	3.10	3.25	0.133	0.11
Spleen	0.21	0.20	0.23	0.21	0.22	0.017	0.77
pH of the digestive tract content							
Stomach	3.30	3.45	3.20	3.00	2.90	0.168	0.17
Jejunum	6.35	6.05	6.10	5.90	5.80	0.219	0.41
Ileum	6.35	6.20	5.85	6.30	5.90	0.298	0.66
Cecum	5.85	5.55	5.50	5.65	5.65	0.139	0.43
Colon	5.95	6.05	6.00	6.15	6.05	0.137	0.91

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and MOS.

3.4. Intestinal morphology

Duodenal VD increased linearly (P<0.05) with the increasing levels of β-glucans, glucomannans, and MOS. The animals fed ANT diet showed the highest (P<0.05) duodenal VD. On the other hand, jejunal

VD did not exhibit a dose-response behavior; however, the inclusion of 2,000 mg kg^{-1} β-glucans, glucomannans, and MOS showed higher VD when compared with the ANT diet (P<0.05). Nevertheless, there was no effect (P>0.05) on the other parameters of intestinal morphology (Table 6).

Table 6 - Villus height (VH, µm), crypt depth (CD, µm), villus height:crypt depth ratio (VH:CD), villus width (VW, µm), and villus density (VD) of the duodenum and jejunum of piglets at 14 d of experiment

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
Duodenum							
VH	187	218	189	198	200	22.887	0.87
CD	83	95	88	92	90	7.408	0.82
VH:CD	2.55	2.45	2.35	2.45	2.55	0.185	0.96
VW	117	107	118	114	116	6.856	0.79
VD[2]	99.22	62.88*	67.55*	75.88*	73.77*	5.036	0.01
Jejunum							
VH	244	249	192	183	232	19.647	0.09
CD	72	89	53	58	75	9.817	0.13
VH:CD	4.05	3.45	4.35	3.65	4.70	0.549	0.55
VW	110	109	91	93	111	8.832	0.31
VD	81.88	74.55	66.00*	94.66*	63.55*	4.141	0.01

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.
[2] Linear effect (P<0.05) of dietary β-glucans, glucomannans, and MOS levels on duodenal villus density ($y = 0.0041x + 63.87$; $R^2 = 0.796$).
* Differs from the antimicrobial treatment by Dunnett's test (P<0.05).

3.5. Intestinal microbiota

Ileal Enterobacteriaceae count, total cecal bacteria count, and cecal lactic acid bacteria count responded quadratically (P<0.05) to the prebiotic levels. The equations estimated the prebiotic inclusion levels of 2,125, 1,167, and 1,500 mg kg^{-1} to provide reduced counts of the respective bacteria (ileal enterobacteria: 6.47 log CFU g^{-1}; total cecal bacteria: 6.70 log CFU g^{-1}; and cecal lactic acid: 7.29 log CFU g^{-1}) (Table 7). *Salmonella* spp. was not detected, whereas *E. coli* and *Lactobacillus* spp. gene copies were unaffected by the dietary treatments (Table 8).

4. Discussion

4.1. Growth performance and diarrhea occurrence

Zhou et al. (2013), who evaluated dietary graded levels up to 100 mg kg^{-1} β-glucans, and Luna et al. (2015), who evaluated a dietary combination of 500 mg kg^{-1} β-glucans and 330 mg kg^{-1} MOS, reported no growth-enhancing effects of dietary β-glucans, glucomannans, and/or MOS on weanling pigs. In contrast to the current results, Shen et al. (2009), who tested the individual dietary inclusion of 5,000 mg kg^{-1} yeast culture, Poeikhampha and Bunchasak (2011), who studied dietary levels up to 2,000 mg kg^{-1} MOS, Thi Tuoi et al. (2016), who tested dietary levels up to 2,000 mg kg^{-1} of a prebiotic based on β-glucans and MOS, and Berto et al. (2020), who evaluated the effects of 4,500 mg kg^{-1} autolyzed yeast (β-glucans, MOS and nucleotides), demonstrated growth-enhancing effects on weanling pigs. In our previous study, Anjos et al. (2019) observed that the increasing dietary levels up to 3,000 mg kg^{-1} of β-glucans, glucomannans, and MOS led to an ascent in FCR of piglets in the nursery phase.

Utiyama et al. (2006), who evaluated dietary MOS levels up to 3,000 mg kg^{-1}, Assis et al. (2014), who studied the alone inclusion of 500 mg kg^{-1} β-glucans and 1,500 mg kg^{-1} MOS, and Luna et al. (2015), who tested the combined inclusion of 500 mg kg^{-1} β-glucans and 330 mg kg^{-1} MOS, reported the

Table 7 - Mean intestinal microbial concentration (log CFU/g) in piglets at 14 d of experiment

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
Jejunum							
Total bacteria	5.53	5.00	5.55	5.53	5.75	0.320	0.32
Lactic bacteria	6.13	5.98	6.55	6.40	6.45	0.366	0.17
Enterobacteria	6.10	5.88	6.54	5.96	5.63	0.311	0.07
Ileum							
Total bacteria	6.95	6.27*	6.79	5.94*	7.08	0.255	0.01
Lactic bacteria	7.60	7.87	7.59	7.10	7.49	0.324	0.57
Enterobacteria[2]	7.26	8.16*	7.37	6.30*	7.18	0.235	0.01
Cecum							
Total bacteria[3]	6.26	7.12*	6.58	6.69	7.16*	0.291	0.04
Lactic bacteria[4]	6.62	7.96*	7.39*	7.36*	7.96*	0.166	0.01
Enterobacteria	5.95	7.46*	7.29*	6.73	7.24*	0.358	0.04
Colon							
Total bacteria	6.58	6.51	6.37	6.51	6.90	0.250	0.66
Lactic bacteria	6.62	7.83*	7.99*	7.82*	8.00*	0.269	0.03
Enterobacteria	6.22	7.16	7.20*	6.11	7.43*	0.338	0.02

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.
[2] Quadratic effect (P<0.05) of dietary β-glucan + MOS levels on the Enterobacteriaceae count in the ileum ($y = 0.0000004x^2 - 0.0017x + 8.2689$, $R^2 = 0.856$).
[3] Quadratic effect (P<0.05) of dietary β-glucan + MOS levels on total bacterial count in the cecum ($y = 0.0000003x^2 - 0.0007x + 7.1066$, $R^2 = 0.984$).
[4] Quadratic effect (P<0.05) of dietary β-glucan + MOS levels on the lactic bacteria count in the cecum ($y = 0.0000003x^2 - 0.0009x + 7.964$, $R^2 = 0.998$).
* Differs from the antimicrobial treatment by Dunnett's test (P<0.05).

Table 8 - Quantification of number of gene copies of *Escherichia coli* and *Lactobacillus* spp. (log10 n of copies/g)

Item	Dietary treatment[1]					SEM	P-value
	ANT	0	1,000	2,000	3,000		
E. coli (*phoA*)							
Ileum	5.83	5.44	5.41	5.94	5.86	0.225	0.94
Cecum	5.89	5.47	5.38	4.41	5.88	0.270	0.57
Lactobacillus spp.							
Ileum	7.76	7.13	8.31	8.14	7.99	0.174	0.16
Cecum	7.78	7.97	8.32	7.55	8.31	0.141	0.39

SEM - pooled standard error of the mean.
[1] ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and MOS.

effectiveness of prebiotics in the prevention of post-weaning diarrhea (PWD) in piglets. Thi Tuoi et al. (2016) observed a lower DO in animals fed a mixture of 1,000 mg kg^{-1} β-glucans + MOS. Those authors related the activity of those additives in the modulation of the intestinal microbiota to the suppressed colonization and proliferation of pathogenic bacteria. However, we did not observe influence on the DO when the piglets received β-glucans, glucomannans, and MOS in their diets.

These inconsistent results might be related to the different animal rearing conditions in the different experiments, such as environmental and bacterial challenges (Cairo et al., 2018; Almeida et al., 2020), health state of the pigs at the beginning of the experimental period (Skinner et al., 2014), ingredient quality and diet complexity (Collins et al., 2017; Wang et al., 2018), experimental period and age of animals (Anjos et al., 2019), different inclusion levels of those additives in the diet (Nochta et al., 2010; Halas and Nochta, 2012) and composition, and method of extraction and presentation forms of the natural bioactive compounds (Brown and Gordon, 2003; Li et al., 2006; Volman et al., 2008).

4.2. Hematological analyses

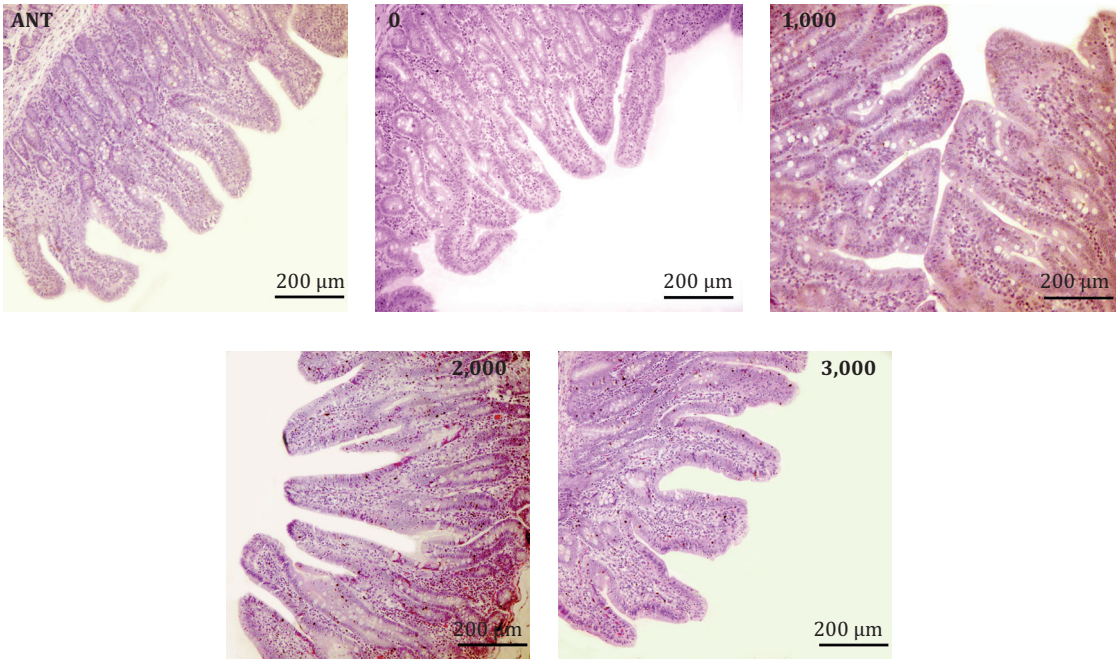
The post-weaning period can influence the hematological parameters in piglets due to several factors such as low feed intake and digestive capacity, PWD caused by exposure to microorganisms, and dietary transition. In addition, the variation in hematological concentration can be affected by sampling time, promoting stress responses with an increase in defense cells, a fact not observed in this study. The main values are within the normal range for swine species (Eze et al., 2010; Saleh et al., 2015), indicating that the animals showed a normal physiological condition during the experiment period.

4.3. Organ weight and pH of digestive tract contents

Overall, piglets maintained the same organ weights and were not influenced by dietary treatments, which apparently demonstrates a normal state of organ development. Santos et al. (2003) and Corassa et al. (2012), also did not observe effects of dietary inclusion of MOS on the pH of the digestive contents or on organ weight in newly weaned piglets. However, Anjos et al. (2019) observed that animals receiving 1,000 and 3,000 mg kg^{-1} of β-glucans, glucomannans, and MOS had a higher relative weight of spleen when compared with those on the negative control treatment. Regarding the authors, these results may be associated with the prebiotic components and composition that can have stimulated of the immune system.

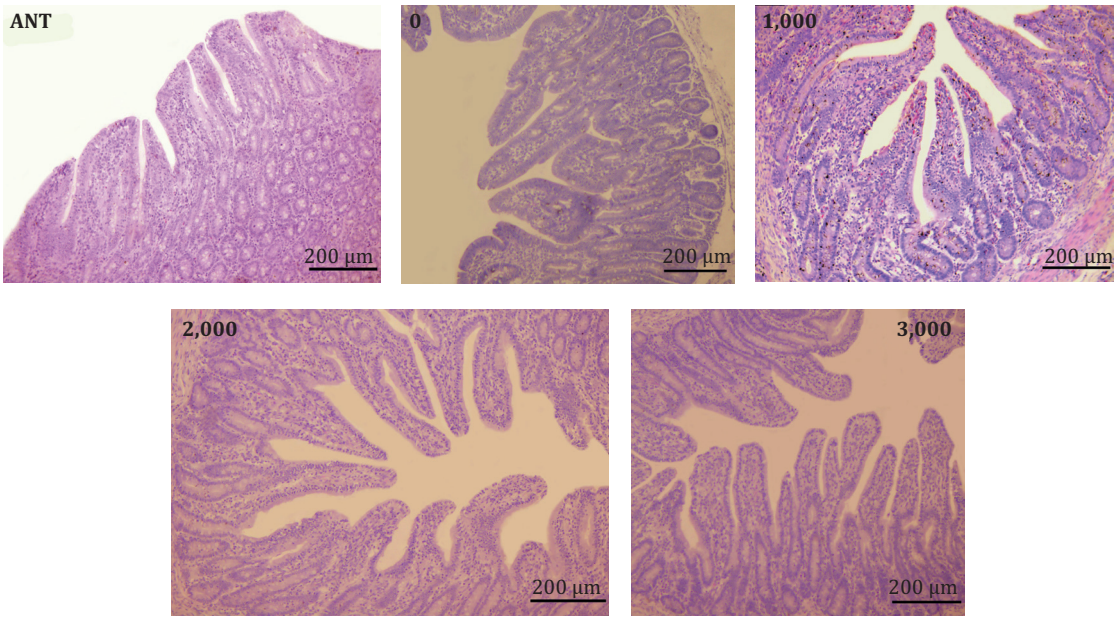
4.4. Intestinal morphology

Intestinal histology was examined in several studies involving piglets. Rigueira et al. (2013) and Tucci et al. (2011) stated that VD is not always correlated with nutrient absorption, whereas enterocyte maturity also plays an essential role in functionality and absorption rate. Such evidence might be an explanation for the lack of significant relations between VD and growth performance in the current study (Figures 1 and 2), since the increase on DO and lower growth performance are related to reduced VH.



ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.

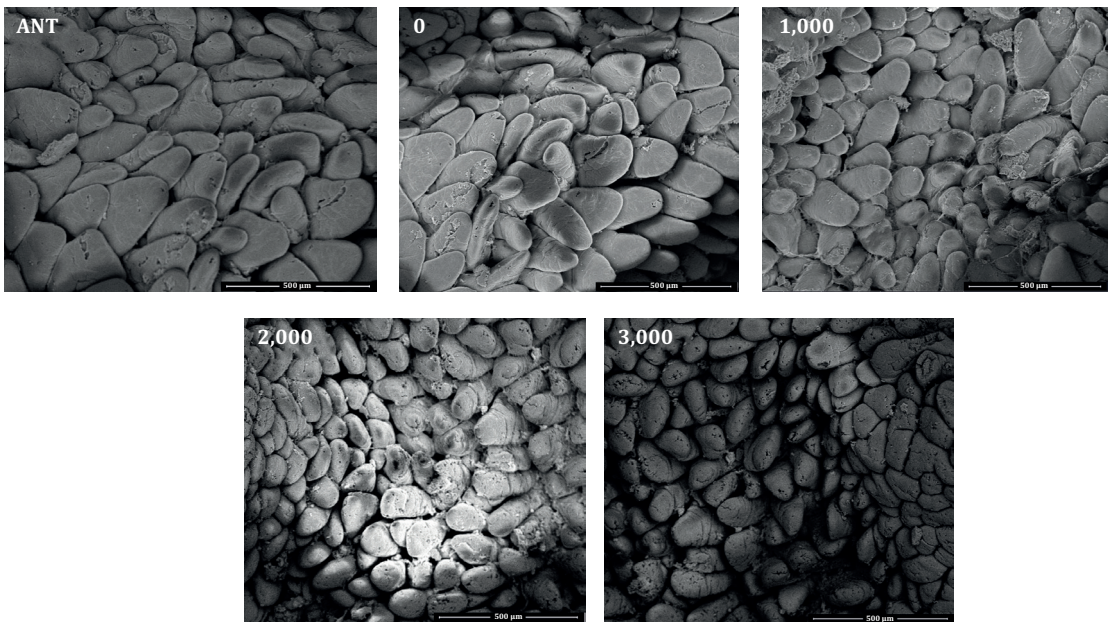
Figure 1 - Microphotographs of duodenum sections of piglets at 14 days of experiment.



ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.

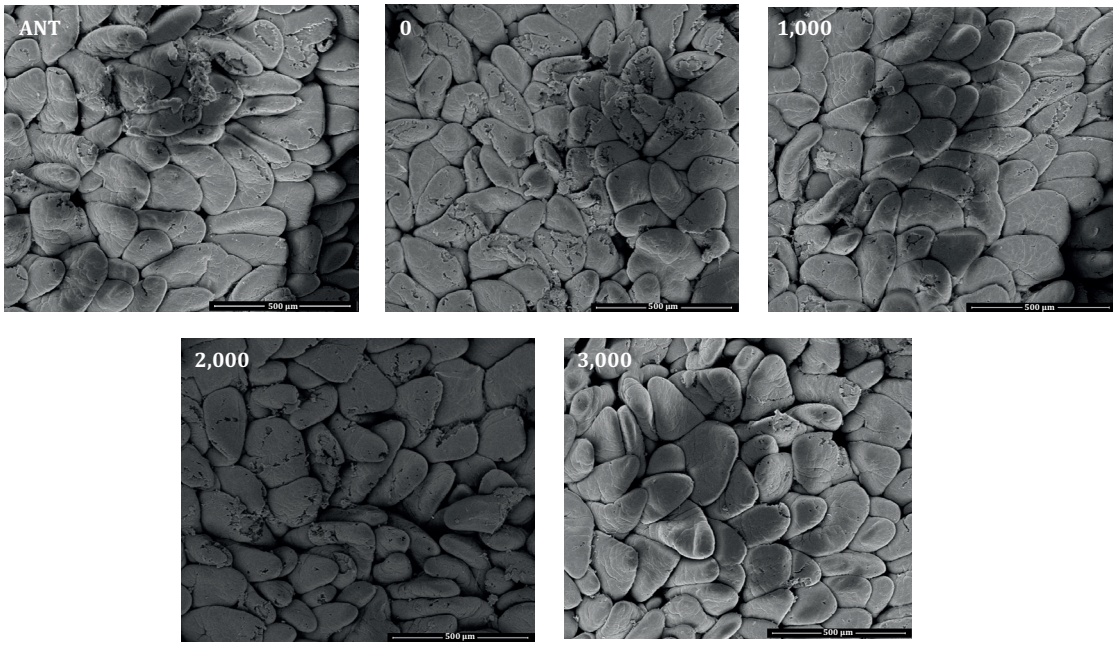
Figure 2 - Microphotographs of jejunum sections of piglets at 14 days of experiment.

However, the pigs fed ANT and 2,000 mg kg^{-1} β-glucans, glucomannans, and MOS diets showed regular villi with a small distance between them and decreased deformities in both duodenum and jejunum (Figures 3 and 4), indicating healthier villi compared with the negative control group.



ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.

Figure 3 - Electromicrographs of the duodenal mucosa of piglets at 14 days of experiment.



ANT - antimicrobial (120 mg kg^{-1} chlorohydroxyquinoline); 0 (negative control); 1,000, 2,000, or 3,000 mg kg^{-1} β-glucans, glucomannans, and mannan-oligosaccharides.

Figure 4 - Electromicrographs of the jejunal mucosa of piglets at 14 days of experiment.

4.5. Intestinal microbiota

The observed reduced lactic acid counts in the cecum and colon without changes in *Lactobacillus* spp. copies in the pigs fed ANT diet might indicate a selective effect of ANT for other genera of lactic acid bacteria such as *Pediococcus*, *Leuconostoc*, *Weisella*, *Lactococcus*, *Streptococcus*, *Enterococcus*, *Tetragenococcus* and *Vagococcus* (Santos et al., 2011).

The effectiveness of antimicrobials and prebiotics in newly weaned piglets is well-documented in the literature (Cromwell, 2002; Bosi et al., 2011; NRC, 2012; Qin et al., 2019). However, this effectiveness may differ depending on rearing conditions, with more evident responses described on commercial farms than in experimental conditions (Niewold, 2007). An explanation for these inconsistent results is the low environmental and bacterial challenge, given the satisfactory sanitary conditions, and the high complexity of the diet (Nochta et al., 2010; Halas and Nochta, 2012). Some authors have proven that greater environmental pressure (challenge) and worsened nutritional conditions may improve the performance of piglets fed additives (oligosaccharides and antimicrobials) used in this experiment (Mao et al., 2005; Li et al., 2006; Price et al., 2010).

Escherichia coli is among the most prevalent microorganisms in piglets' intestinal tract microbiota, and it can be isolated in healthy or sick animals. Pathogenic strains are of great importance in pig farming, as they can cause enteritis and PWD. What differentiates the native strain from the pathogenic one is the presence of virulence factors in the latter (Maciel et al., 2018). However, even non-pathogenic strains of *E. coli* can cause infections in immunosuppressed or debilitated animals (Nataro and Kaper, 1998).

The present results corroborate those reported by Poeikhampha and Bunchasak (2011), who used 2,000 mg kg^{-1} MOS in the diet of weaned piglets and did not observe a reduction in the *E. coli* count in the cecum and rectum of the animals. Conversely, Zhou et al. (2013) used 1,000 mg kg^{-1} β-glucans and found a marked reduction in the *E. coli* count in piglet feces. Additionally, Stuyven et al. (2009) reported that piglets fed a diet containing 500 mg kg^{-1} β-glucans for two weeks post-weaning were

less susceptible to infection by enterotoxigenic *E. coli*, exhibiting a marked reduction in the excretion of this pathogen in the feces.

5. Conclusions

Dietary inclusion of a prebiotic based on β-glucan, glucomannan, and mannan-oligosaccharides or antibiotic (chlorohydroxyquinoline) did not enhance growth performance or intestinal health of pigs during the two first weeks post-weaning. The slight effects observed on villus density and integrity and microbiota profile might be an indication of the potential benefits of those products in commercial farming conditions.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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