DESENVOLVIMENTO SOCIAL E SUSTENTÁVEL DAS CIÊNCIAS AGRÁRIAS

Júlio César Ribeiro
(Organizador)

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APRESENTAÇÃO

O desenvolvimento sustentável das Ciências Agrárias assegura um crescimento socioeconômico satisfatório reduzindo potenciais impactos ambientais, ou seja, proporciona melhores condições de vida e bem estar sem comprometer os recursos naturais.

Neste contexto, a obra "Desenvolvimento Social e Sustentável das Ciências Agrárias" em seus 3 volumes traz à luz, estudos relacionados a essa temática.

Primeiramente são apresentados trabalhos a cerca da produção agropecuária, envolvendo questões agroecológicas, qualidade do solo sob diferentes manejos, germinação de sementes, controle de doenças em plantas, desempenho de animais em distintos sistemas de criação, e funcionalidades nutricionais em animais, dentre outros assuntos.

Em seguida são contemplados estudos relacionados a questões florestais, como características físicas e químicas da madeira, processos de secagem, diferentes utilizações de resíduos madeireiros, e levantamentos florestais.

Na sequência são expostos trabalhos voltados à educação agrícola, envolvendo questões socioeconômicas e de inclusão rural.

O organizador e a Atena Editora agradecem aos autores por compartilharem seus estudos tornando possível a elaboração deste e-book.

Esperamos que a presente obra possa contribuir para novos conhecimentos que proporcionem o desenvolvimento social e sustentável das Ciências Agrárias.

Boa leitura!

Júlio César Ribeiro

SUMÁRIO

CAPÍTULO 14

EFFECTS OF YEAST CELL WALL ASSOCIATED WITH ORGANIC ACID BLEND ON POST-WEANING DIARRHEA AND PERFORMANCE IN PIGLETS

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ABSTRACT: The objective of this study was to evaluate the effects of the yeast cell wall (YCW) associated with a blend of organic acids (OA) supplemented in the diets of piglets during the nursery phase. 900 male and female post-weaning piglets (Danbred x Piétrain) with 25 days of age were distributed into three treatments: 1- Control (without supplementation); 2 – YCW+OA 7 g/kg (blend of 19% benzoic acid, 15% sodium benzoate, 25% formic acid, 4% phosphoric acid, 4.5% citric acid and 15% YCW of *Saccharomyces cerevisiae*); 3 – YCW+OA 14 g/kg (same composition as treatment 2). Each treatment had 12 replicates of 25 animals each. The diets were divided into initial (from 25 to 38 days) and growth (from 39 to 66 days). Feed intake (FI, kg), body weight (BW, kg), body weight gain (BWG, kg), and feed conversion ratio (FCR) at 38 and 66 days were evaluated. The mortality and feces score were observed daily. The data were analyzed by the PROC GLM of the SAS at 5% significance, and the difference between the averages compared by the Tukey test. It was observed effects of YCW+OA supplementation over piglets performance between 25 and 66 days, where the supplementation of 7 g/kg and 14 g/kg increased by 5.2% and 6.7%, the BWG ($P<0.05$), and improved the FCR by 4.1% and 6.1% ($P <0.05$), respectively, when compared to the control group. There was also a significant improvement in the feces score for both treatments with YCW+OA compared to the control group ($P<0.05$). However, no statistical difference in mortality between groups ($P> 0.05$) was observed. Both doses of YCW+OA had a positive impact on performance and reduced diarrhea in post-weaned piglets.

KEYWORDS: *Saccharomyces cerevisiae,* nutrition, nursery period.

EFEITOS DA PAREDE CELULAR DE LEVEDURA ASSOCIADA À ÁCIDOS ORGÂNICOS SOBRE A INCIDÊNCIA DE DIARRÉIA PÓS DESMAME E DESEMPENHO DE LEITÕES

RESUMO: O objetivo do estudo foi avaliar os efeitos da parede celular de levedura (PCL) associados à um blend de ácidos orgânicos (AO) suplementados nas dietas de leitões em fase de creche. Para isso foram utilizados 900 leitões machos e fêmeas pós-desmame (Danbred x Piétrain) com 25 dias de idade que foram distribuídos três tratamentos: 1- Controle (não suplementado); 2 - PCL+AO 7 g/kg (blend de 19% de ácido benzóico, 15% de benzoato de sódio, 25% de ácido fórmico, 4% de ácido fosfórico, 4,5% de ácido cítrico e 15% de PCL de *Saccharomyces cerevisiae*); 3 - PCL+AO 14 g/kg (mesma composição do tratamento 2). Cada tratamento tinha 12 repetições de 25 animais cada. As dietas foram divididas em inicial (de 25 a 38 dias) e crescimento (de 39 a 66 dias). Foram avaliados consumo de ração (CR, kg), peso corporal (PC, kg), ganho de peso (GP, kg) e conversão alimentar (CA) aos 38 e 66 dias; já a mortalidade e o escore das fezes foram observadas diariamente. Os dados foram analisados pelo PROC GLM do SAS a 5% de significância, sendo a diferença entre as médias estabelecida pelo teste Tukey. Foram observados efeitos da suplementação de PCL+AO sobre o desempenho dos leitões entre 25 e 66 dias de idade, onde a suplementação de 7 g/kg e 14 g/kg aumentou, respectivamente, 5,2% e 6,7% o GP quando comparado ao grupo controle ($P < 0,05$) e melhorou a CA em 4,1% e 6,1% ($P<0,05$), respectivamente. Houve também melhora significativa no escore de fezes para ambos os tratamentos com PCL+AO comparados ao grupo controle ($P<0,05$). Não houve diferença estatística em mortalidade entre os grupos ($P>0,05$). Ambas as doses de PCL+AO tiveram impacto positivo sobre o desempenho e redução de diarreia em leitões em fase de creche.

PALAVRAS-CHAVE: *Saccharomyces cerevisiae,* nutrição, fase de creche.

1 I INTRODUCTION

The weaning is a challenging period for the piglet, and the stressor factors involve, mainly, the new environment with new social interaction and change of diet. The pigs are handling and regrouped many times during their productive live (MARTÍNEZ-MIRÓ et al. 2016); however, during the weaning period, the consequences of this stress can lead to high production losses. After the birth, the piglet is highly dependent on specific and non-specific immune factors present in maternal colostrum, and milk (STOKES et al., 2004), and the commercial weaning occurs between 17 to 28 days of age when the piglet immune system is still immature, and their circulating antibodies reach the lowest levels (around 28 days). This period is called the “immunity gap” or “post-weaning gap,” where the piglet is more susceptible to intestinal challenges because their acquired immune system has not had time to develop fully. After that, the antibody level gradually increases as the animal builds its natural immunity.

At the same time, the piglet digestive system has some limitations, such as insufficient secretion of enzymes, hydrochloric acid, bicarbonate and mucus, factors that interfere with proper digestion and absorption of nutrients (LALLÈS et al., 2007). The stress of change milk (high-digestible) to solid feed (less-digestible more-complex feed) can result in a decrease in feed intake and water. According to Brooks and Beal (2001), 50% of weaned piglets consume the feed until 24 hours post-weaning, and 10% starts to eat 48 hours post-weaning. The lower digestibility of the diet (depending on the quality of the ingredients used) can be used as a substrate for pathogenic bacteria proliferation and result in health and enteric problems, such as diarrhea. In this period, the intestinal microbiota leads to dramatic changes in the composition during the 7–14 days after weaning (HILLMAN, 2001) and should generate resistance or competitive exclusion (LALLÈS et al., 2007). Also, piglets have high intestinal permeability, which results in higher susceptibility to contamination, such as the luminal translocation of bacteria, toxins, and antigens to the subepithelial tissues. All these factors together can lead to systemic inflammatory activation of the intestinal mucosa, causing several problems.

Considering this scenario, the antibiotics and other chemicals have been used for several decades to control enteric problems in post-weaning piglets; however, it impacts gut microbiota has been more recently investigated, and researchers have shown that, in addition to altering the composition of the microbiota, antibiotics also can affect the gene expression, protein activity and the overall metabolism of the intestinal microbiota. Microbial changes caused by antibiotics increase the immediate risk of infection, and can also affect basic immunological homeostasis in the long-term. Thus, the use of high-quality natural additives, free of contaminants, with a focus on intestinal health and animal immunity is essential to keep the farm sustainable and profitable in the future.

There are several alternatives available to control pathogenic bacteria and enteric problems, such as probiotics, organic acids, plant extracts, prebiotics, etc. Each product has a different mode of action, directly or indirectly, modulating the microbiota and the response of the immune system. The yeast cell wall from *Saccharomyces cerevisiae* is classified as prebiotic (indigestible functional fiber) mainly composed of mannan-oligosaccharides (MOS) and β-glucans. MOS acts preventing the pathogen colonization in the gut as it offers a binding site to harmful bacteria that possess *fimbria*, then the "trapped" bacteria will be excreted together with the fecal material. The β-glucans can modulate the immune responses as they are natural stimulants of the innate immune system. When phagocytic cells are in contact with β-glucans, these cells are stimulated, and cytokines are produced. The production of cytokines will trigger a "chain reaction," inducing a higher immune status in animals, making them able to resist or fight better against opportunistic infections.

The organic acids category are weak short-chain acids (C1 - C7) that have a constant dissociation (pKa) of 3 to 5 (VIOLA, 2006), which is dependent on the pH of the medium. Therefore, the compartment of the digestive tract will vary, and the acid will be able to dissociate and have its mechanism of action or not. The mechanism of action occurs, reducing the pH in the media that inhibits or stops the growth and proliferation of microorganisms, and the non-dissociated form of the acid. That format can cross the microorganism cell membrane and then dissociate into the cytoplasm, reducing intracellular pH, altering metabolism, and increasing osmotic pressure, leading to cell disruption. The efficiency of this process is linked to the size of the acid chain and whether it has a hydrophobic characteristic to cross the cell membrane (VIOLA, 2006; BELLAVER and SCHEUERMANN, 2004). Short-chain acids can also be absorbed by intestinal cells and used as an energy source and may have an indirect trophic action on the villi.

Thus, the objective of this study was to evaluate the effects of the yeast cell wall (YCW) associated with a blend of organic acids (OA) supplemented in the diets of piglets during the nursery phase housed in a commercial pig farm with a high incidence of *E. coli* associated post-weaning diarrheas.

2 | MATERIAL AND METHODS

The study was performed according to the guidelines according to EFSA administrative/technical guidance, updated September 2014 and Commission Regulation (EC) Nº 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) Nº 1831/2003.

The Freie Universität Berlin conducted the trial at a commercial pig breeding farm in Germany, where a total of 900 post-weaning barrows and gilts (Danbred x Piétrain) without visual evidence of clinical signs were selected from a pool of about 1020 piglets (age at weaning: 25 ± 03 days) and were allotted equally according to the body weight, litter, and gender at random to 36 pens (25 piglets per pen), equipped with solid partitions and slotted floors within a post-weaning house. Before weaning, piglets had access to creep feed in mash form. Three treatments were evaluated: 1 - Control (without supplementation); 2 – YCW+OA 7 g/kg (blend of 15% YCW of *Saccharomyces cerevisiae* – product named ImmunoWall®, from ICC Brazil company + 19% benzoic acid, 15% sodium benzoate, 25% formic acid, 4% phosphoric acid, 4.5% citric acid – product named ImmunoAcid®, from FF Chemicals company); 3 – YCW+OA 14 g/kg (same composition as treatment 2).

Throughout the 42-d feeding period, the house temperature, relative humidity, lighting, and forced ventilation was measured daily and, if necessary, adjusted to the targeted values. Environmental and management conditions were under targets used in commercial pig breeding farms. The average house temperature was kept at about 29 °C during the first week after weaning. From the second week after weaning onwards, the house temperature was stepwise reduced up to about 22 °C from week 5 after weaning onwards. The relative humidity was within the range of 55 to 65%.

The lighting regime (natural/artificial) consisted of a 16h light- (about 40 lux) and 8h dark-cycle. Post-weaning piglets had *ad libitum* access to feed (mash form); water supplied by drinking bowls was also available *ad libitum*. All daily management tasks were performed starting with pigs fed Control diets without supplementation of YCW+OA, to eliminate potential handling related carry-over.

The 42-d feeding period was divided into two feeding phases; a starter (25 to 38 days of age) and a subsequent grower diet (39 to 66 days of age), meeting or slightly exceeding the nutritional requirements of post-weaning piglets as recommended by the Society of Nutrition Physiology (2006) except for calcium. The basal mixtures containing no further feed additives such as enzymes or probiotics and the concentrations of zinc and copper were at adequate but not at excess levels to avoid the potential confounding effect of YCW+OA. The composition of the diets is given in Tables 1 and 2, respectively.

Treatment groups		**Control**	**YCW+AO 7g/kg**	**YCW+AO 14g/kg**
Ingredients				
Corn	%	45.00	45.00	45.00
Soybean meal (CP:49%)	%	19.60	19.60	19.60
Barley	%	15.00	15.00	15.00
Skim milk powder	%	10.00	10.00	10.00
Wheat	%	4.18	4.18	4.18
Premix [1]	%	1.20	1.20	1.20
Limestone	%	1.00	0.50	-----
Soybean oil	%	0.90	0.90	0.90
Monocalcium phosphate	%	0.80	0.80	0.80
L-Lysine-HCL	%	0.50	0.50	0.50
DL-Methionine	%	0.19	0.19	0.19
L-Threonine	%	0.16	0.16	0.16
L-Tryptophan	%	0.07	0.07	0.07
Titanium(IV)dioxide	%	-----	0.50	1.00
Corn starch	%	1.40	0.70	-----
YCW+AO [2]	%	-----	0.70	1.40

Nutritional composition				
ME [3]	MJ/kg	13.62	13.62	13.62
Crude protein	%	20.05	20.05	20.05
Lysine	%	1.45	1.45	1.45
Methionine	%	0.53	0.53	0.53
Methionine & Cysteine	%	0.84	0.84	0.84
Threonine	%	0.92	0.92	0.92
Tryptophan	%	0.28	0.28	0.28
Crude fat	%	3.47	3.21	3.00
Crude fiber	%	2.66	2.67	2.66
Crude ash	%	4.90	4.90	4.90
Calcium	%	0.72	0.65	0.55
Phosphorus	%	0.61	0.62	0.63
Available phosphorus	%	0.35	0.35	0.35
Sodium	%	0.22	0.22	0.22

Table 1. Composition of the starter diets from 25 to 38 days of age (as-fed). [1)] Contents per kg Premix: 400000 I.U. vit. A (acetate); 120000 I.U. vit. D_3; 8000 mg vit. E (α-tocopherole acetate); 200 mg vit. K_3 (MSB); 250 mg vit. B_1 (mononitrate); 420 mg vit. B_2 (cryst. riboflavin); 2500 mg niacin (niacinamide); 400 mg Vit. B_6 (HCl); 2000 μg vit. B_{12}; 25000 mg Biotin (commercial, feed grade); 1000 mg pantothenic acid (Ca d-pantothenate); 100 mg folic acid (cryst. commercial feed grade); 80000 mg choline (chloride); 5000 mg Zn (sulfate); 5000 mg Fe (carbonate); 6000 mg Mn (sulfate); 1000 mg Cu (sulfate-pentahydrate); 20 mg Se (Na-selenite); 45 mg J (Ca-iodate); 130 g Na (NaCl); 55 g Mg (sulfate); [2)] Calculated by using the estimation given by DLG 2013. [3)] YCW+OA : ImmunoAcid®.

Treatment groups		Control	YCW+AO 7g/kg	YCW+AO 14g/kg
Ingredients				
Corn	%	50.00	50.00	50.00
Soybean meal (CP:49%)	%	23.15	23.15	23.15
Barley	%	10.60	10.60	10.60
Wheat	%	10.00	10.00	10.00
Premix [1]	%	1.20	1.20	1.20
Limestone	%	1.00	0.50	-----
Monocalcium phosphate	%	1.00	1.00	1.00
Soybean oil	%	0.85	0.85	0.85
L-Lysine-HCl	%	0.48	0.48	0.48
DL-Methionine	%	0.14	0.14	0.14
L-Threonine	%	0.13	0.13	0.13
L-Tryptophan	%	0.05	0.05	0.05
Titanium(IV)dioxide	%	-----	0.50	1.00

Corn starch	%	1.40	0.70	-----
YCW+AO [2]	%	-----	0.70	1.40
Nutritional composition				
ME [3]	MJ/kg	13.54	13.54	13.54
Crude protein	%	19.00	19.00	19.00
Lysine	%	1.30	1.30	1.30
Methionine	%	0.43	0.42	0.42
Methionine & Cysteine	%	0.75	0.75	0.75
Threonine	%	0.83	0.83	0.83
Tryptophan	%	0.25	0.25	0.25
Crude fat	%	3.64	3.36	3.12
Crude fiber	%	2.78	2.80	2.81
Crude ash	%	4.57	4.32	4.08
Calcium	%	0.63	0.54	0.45
Phosphorus	%	0.59	0.60	0.59
Available phosphorus	%	0.32	0.32	0.32
Sodium	%	0.20	0.20	0.20

Table 2. Composition of the grower diets from 39 to 66 days of age (as-fed). [1)] Contents per kg Premix: 400000 I.U. vit. A (acetate); 120000 I.U. vit. D_3; 8000 mg vit. E (α-tocopherole acetate); 200 mg vit. K_3 (MSB); 250 mg vit. B_1 (mononitrate); 420 mg vit. B_2 (cryst. riboflavin); 2500 mg niacin (niacinamide); 400 mg Vit. B_6 (HCl); 2000 µg vit. B_{12}; 25000 µg Biotin (commercial, feed grade); 1000 mg pantothenic acid (Ca d-pantothenate); 100 mg folic acid (cryst. commercial feed grade); 80000 mg choline (chloride); 5000 mg Zn (sulfate); 5000 mg Fe (carbonate); 6000 mg Mn (sulfate); 1000 mg Cu (sulfate-pentahydrate); 20 mg Se (Na-selenite); 45 mg J (Ca-iodate); 130 g Na (NaCl); 55 g Mg (sulfate); [2)] Calculated by using the estimation given by DLG 2013. [3)] YCW+OA : ImmunoAcid®..

The piglet's body weight per pen and the amount of feed supplied were measured at the initial day of the 42-d feeding period and the end of each following week. The individual body weight gain was calculated using the mean body weight per pen at the end of each period, minus the averaged body weight per pen at the start of each period. Feed intake per piglet was estimated as the total amount of feed supplied per pen and period corrected by dispersed feed and leftovers, and the number of piglets per pen. The feed conversion ratio was calculated on the relationship of weekly adjusted feed intake per pen and body weight gain per pen for this period.

All piglets were monitored twice a day throughout the 42-d experimental period for any abnormalities, abnormal behavior, and fecal consistency. The appearance of feces (scoring) was daily scored per pen and summarized in weekly intervals per pen as follows: 1 - Liquid diarrhea; 2 - Pasty feces falling out of shape upon contact with surfaces; 3 - Formed feces, soft to cut; 4 - Well-formed feces, firm to cut, but not dry; 5 - Hard and dry feces. The incidence rate of post-weaning diarrhea and other disorders was expressed as the ratio of the number of diseased piglets to the total number of pigs in the treatment group by the time of weaning. The mortality rate was calculated as the percentage ratio of piglet's dead to the total number of pigs in the treatment group at weaning. Finally, antibiotic treatments used (below) were recorded:

- Baytril® (enrofloxacin: 2.5 mg/ kg body weight /d) mainly in cases of post-weaning diarrhea and exudative dermatitis; one-time application using intramuscular injection;

- Hostamox® (amoxicillin: 15 mg/kg body weight/d) in cases of respiratory disorders; three-time application using intramuscular injection every 24h;

- Metacam® (meloxicam: 0.2 mg/kg body weight/d); one-time using intra-muscular injection.

The statistical model used the fixed effect of treatment and for the body weight the pen as a random factor. The statistical analyses were performed with the software package SPSS (IBM SPSS Version 21) and based on one-way ANOVA. All treatment means were compared with each other, and the Tukey adjustment was used to control the family-wise error rate. Differences among means with a probability of $P<0.05$ were accepted as statistically significant; mean differences with P-values ranging from 0.05 to 0.10 were accepted as trends.

3 | RESULTS AND DISCUSSION

The trial was run without any adverse technical events (power failure, feed/ water failures, etc.). Diseases, antibiotic treatments, and mortality in post-weaned piglets recorded throughout the 42-d feeding period are summarized in Table 3.

Treatment groups		**Control**	**YCW+AO 7g/kg**	**YCW+AO 14g/kg**
Diseased piglets				
Post-weaning diarrhea	nº	45	23	17
Respiratory disorders	nº	25	19	18
Other diseases	nº	7	8	9
Antibiotic treatments (body temperature > 39 °C				
Post-weaning diarrhea	nº	35	18	11
Respiratory disorders	nº	20	14	16
Other diseases	nº	5	6	6

Cumulative mortality				
Mortality total	nº	8	4	2
Mortality rate	%	2.70	1.33	0.67

Table 3. Diseases, mortality, and treatments in post-weaning piglets from 01 to 42 days on trial (25 to 66 days of age).

The diseases were mainly determined by post-weaning diarrhea and reflected the expected common problem in the selected pig farm. Furthermore, respiratory diseases were found characterized by coughing, sneezing, and in few cases, combined with abdominal breathing. Other conditions included mainly signs of skeletal disorders. The total incidence rate in piglets fed diets without YCW+OA amounted to 25.7% (77 piglets) whereby 77.9% of the diseased piglets were treated with antibiotics as an injectable in combination with an anti-inflammatory agent. The incidence rate of piglets fed diets containing YCW+OA reached 16.7 and 14.7%, respectively. In consequence, antibiotic treatments were up to 45% lower than those recorded in the control group.

Based on bacteriological investigations in feces, the most dominant microbial species associated with post-weaning diarrhea were toxicogenic *E. coli*. The mortality in both treatments was due to post-weaning diarrhea, caused by toxin-producing *E. coli*., and occurred at the end of the first week and during the second week after weaning. The lower incidence rate of post-weaning diarrhea in piglets fed diets containing the YCW+OA resulted in a reduced mortality rate (7g/kg: 2.77% vs. 1.33%; 14 g/kg: 2.77% vs. 0.67%).

Performance parameters of post-weaning piglets recorded from 25 to 66 days of age (42-d feeding period) are presented either weekly or for the different feeding phases in Tables 4 and 5, respectively. All measured values among treatment groups were normally distributed.

Treatment groups		Control	YCW+AO 7g/kg	YCW+AO 14g/kg	P-value
01 to 07 days on trial (25 to 31 days of age)					
Body weight					
- start	kg	6.57 ± 0.32	6.56 ± 0.37	6.56 ± 0.36	0.995
- end	kg	6.91 ± 0.38	7.07 ± 0.36	7.11 ± 0.33	0.356
Weight gain	kg	0.35 ± 0.15[a]	0.51 ± 0.12[b]	0.55 ± 0.10[b]	<0.001
Feed intake	kg	0.42 ± 0.19[a]	0.58 ± 0.14[b]	0.62 ± 0.11[b]	0.005
Feed conversion[1)]		1.218 ± 0.076[a]	1.128 ± 0.050[b]	1.123 ± 0.052[b]	0.001

08 to 14 days on trial (32 to 38 days of age)					
Body weight					
- start	kg	6.91 ± 0.38	7.07 ± 0.36	7.11 ± 0.33	0.356
- end	kg	8.31 ± 0.43[a]	8.73 ± 0.43[ab]	8.83 ± 0.29[b]	0.006
Weight gain	kg	1.40 ± 0.31[a]	1.66 ± 0.23[b]	1.72 ± 0.34[b]	0.032
Feed intake	kg	1.74 ± 0.37	1.94 ± 0.28	1.99 ± 0.38	0.208
Feed conversion[1)]		1.245 ± 0.052[a]	1.170 ± 0.067[b]	1.158 ± 0.051[b]	0.001
15 to 21 days on trial (39 to 45 days of age)					
Body weight					
- start	kg	8.31 ± 0.43[a]	8.73 ± 0.43[b]	8.83 ± 0.29[b]	0.006
- end	kg	10.60 ± 0.42[a]	11.41 ± 0.48[b]	11.59 ± 0.48[b]	<0.001
Weight gain	kg	2.29 ± 0.28[a]	2.69 ± 0.30[b]	2.76 ± 0.43[b]	0.004
Feed intake	kg	3.16 ± 0.35	3.39 ± 0.40	3.34 ± 0.47	0.374
Feed conversion[1)]		1.391 ± 0.149[a]	1.262 ± 0.058[b]	1.211 ± 0.066[b]	<0.001
22 to 28 days on trial (46 to 52 days of age)					
Body weight					
- start	kg	10.60 ± 0.42[a]	11.41 ± 0.48[b]	11.59 ± 0.48[b]	<0.001
- end	kg	13.88 ± 0.65[a]	14.90 ± 0.46[b]	15.11 ± 0.54[b]	<0.001
Weight gain	kg	3.28 ± 0.43	3.49 ± 0.47	3.51 ± 0.28	0.301
Feed intake	kg	4.79 ± 0.52	4.94 ± 0.69	4.83 ± 0.41	0.778
Feed conversion[1)]		1.466 ± 0.074[a]	1.418 ± 0.053[ab]	1.376 ± 0.069[b]	0.008
29 to 35 days on trial (53 to 59 days of age)					
Body weight					
- start	kg	13.88 ± 0.65[a]	14.90 ± 0.46[b]	5.11 ± 0.54[b]	<0.001
- end	kg	18.25 ± 0.64[a]	19.33 ± 0.54[b]	19.58 ± 0.67[b]	<0.001
Weight gain	kg	4.37 ± 0.29	4.43 ± 0.31	4.48 ± 0.34	0.713
Feed intake	%	6.53 ± 0.37	6.38 ± 0.35	6.33 ± 0.46	0.417
Feed conversion[1)]	kg	1.496 ± 0.044[a]	1.442 ± 0.053[b]	1.414 ± 0.040[b]	<0.001
36 to 42 days on trial (60 to 66 days of age)					
Body weight					
- start	kg	18.25 ± 0.64[a]	19.33 ± 0.54[b]	19.58 ± 0.67[b]	<0.001
- end	kg	23.17 ± 0.62[a]	24.36 ± 0.53[b]	24.68 ± 0.65[b]	<0.001
Weight gain	kg	4.91 ± 0.36	5.03 ± 0.22	5.10 ± 0.65	0.257
Feed intake	kg	7.91 ± 0.46	7.83 ± 0.41	7.81 ± 0.37	0.831
Feed conversion[1]		1.612 ± 0.081[a]	1.558 ± 0.045[b]	1.532 ± 0.058[b]	0.012

Table 4. Effects of Yeast Cell Wall + Organic Acid blend (YCW+OA) on performance in post-weaning piglets from 25 to 66 days of age (01 to 42 days on trial). [1)] kg feed per kg body weight gain; [ab] Means with different superscripts within the row differ significantly ($P<0.05$).

Treatment groups		Control	YCW+AO 7g/kg	YCW+AO 14g/kg	P-value
Starter period from 01 to 14 days on trial (25 to 38 days of age)					
Body weight					
- start	kg	6.57 ± 0.32	6.56 ± 0.37	6.56 ± 0.36	0.995
- end	kg	8.31 ± 0.43[a]	8.73 ± 0.43[ab]	8.83 ± 0.29[b]	0.006
Weight gain	kg	1.74 ± 0.37[a]	2.17 ± 0.28[b]	2.27 ± 0.40[b]	0.002
Daily weight gain	g	124 ± 26[a]	155 ± 20[b]	162 ± 29[b]	0.002
Feed intake	kg	2.16 ± 0.46[a]	2.52 ± 035[ab]	2.61 ± 0.46[b]	0.034
Daily feed intake	g	154 ± 33[a]	180 ± 25[b]	186 ± 33[b]	0.034
Feed conversion[1)]		1.238 ± 0.046[a]	1.159 ± 0.048[b]	1.149 ± 0.043[b]	<0.001
Grower period from 15 to 42 days on trial (39 to 66 days of age)					
Body weight					
- start	kg	8.31 ± 0.43[a]	8.73 ± 0.43[ab]	8.83 ± 0.29[b]	0.006
- end	kg	23.17 ± 0.62[a]	24.36 ± 0.53[b]	24.68 ± 0.65[b]	<0.001
Weight gain	kg	14.85 ± 0.59[a]	15.63 ± 0.66[b]	15.85 ± 0.72[b]	0.002
Daily weight gain	g	530 ± 21[a]	558 ± 24[b]	566 ± 26[b]	0.002
Feed intake	kg	20.63 ± 0.49	20.81 ± 0.75	20.69 ± 0.68	0.793
Daily feed intake	g	737 ± 18	743 ± 27	739 ± 24	0.793
Feed conversion[1)]		1.391 ± 0.057[a]	1.333 ± 0.049[b]	1.306 ± 0.050[b]	0.001
Overall period from 01 to 42 days on trial (25 to 66 days of age)					
Body weight					
- start	kg	6.57 ± 0.32	6.56 ± 0.37	6.56 ± 0.36	0.995
- end	kg	23.17 ± 0.62[a]	24.36 ± 0.53[b]	24.68 ± 0.65[b]	<0.001
Weight gain	kg	16.60 ± 0.54[a]	17.80 ± 0.58[b]	18.12 ± 0.84[b]	<0.001
Daily weight gain	g	395 ± 13[a]	424 ± 14[b]	432 ± 20[b]	<0.001
Feed intake	kg	22.79 ± 0.83	23.32 ± 0.84	23.29 ± 1.02	0.275
Daily feed intake	g	543 ± 20	555 ± 20	555 ± 24	0.275
Feed conversion[1)]		1.374 ± 0.046[a]	1.311 ± 0.045[ab]	1.286 ± 0.038[b]	0.001

Table 5. Effects of Yeast Cell Wall + Organic Acid blend (YCW+OA) on performance in post-weaning piglets during the different feeding phases from 25 to 66 days of age (01 to 42 days on trial). *[1)] kg feed per kg body weight gain; [ab] Means with different superscripts within the row differ significantly (P<0.05).*

The initial body weight of the selected post-weaning piglets (gender, litter) reached, on average, 6.56 kg, and was nearly similar in all treatment groups (P>0.05). The body weight at the end of the 42-d feeding period, in piglets, fed diets without the addition of YCW+OA, amounted to 23.17 kg; which was corresponding to an overall body weight gain of 16.60 kg or approximately 395 g per day. When feeding piglets with diets containing YCW+OA at 7 or 14 g/kg levels, the overall body weight gain was significantly (P<0.05) enhanced by 7.2% and 9.2% when compared to the control group. These significant benefits were mainly a result of the statistically relevant higher body weight gain during the starter period in comparison to the control. Differences between the 7 and 14 g/kg of YCW+OA doses were without statistical (P>0.05) relevance.

The overall feed intake of piglets offered diets without the addition of YCW+OA amounted to 22.79 kg or 543 g per day. Piglets fed diets with supplementation of YCW+OA showed, on average, a slightly numerical (P>0.05) higher feed intake than those supplied diets control (+2.3%). Differences between 7 and 14 g/kg levels were not evident.

The calculated overall feed conversion ratio of piglets fed diets control reached 1.374. In piglets fed diets supplemented with YCW+OA at the 7 or 14 g/kg a slightly (-4.5%) or significantly (-6.3%) reduced overall feed conversion ratio in comparison to the control group was found. Nearly corresponding effects were recorded during the starter and grower period.

The results of the scoring of piglet feces were summarized in weekly periods and presented in Table 6. Measured values among treatment groups were normally distributed.

Treatment groups		**Control**	**YCW+AO 7g/kg**	**YCW+AO 14g/kg**	**P-value**
Weekly					
Faecal scoring [1]					
- 01 to 07 d on trial	nº	3.63 ± 0.15[a]	3.73 ± 0.11[ab]	3.80 ± 0.07[b]	0.004
- 08 to 14 d on trial	nº	3.60 ± 0.13[a]	3.79 ± 0.11[b]	3.80 ± 0.07[b]	<0.001
- 15 to 21 d on trial	nº	3.81 ± 0.12[a]	3.93 ± 0.15[b]	3.97 ± 0.07[b]	0.002
- 22 to 28 d on trial	nº	3.94 ± 0.10	3.98 ± 0.06	3.98 ± 0.06	0.326
- 29 to 35 d on trial	nº	3.96 ± 0.08	3.96 ± 0.08	3.98 ± 0.06	0.856
- 36 to 42 d on trial	nº	3.94 ± 0.08	3.95 ± 0.09	3.98 ± 0.06	0.536
Starter period from 01 to 14 days on trial (25 to 38 days of age)					
Faecal scoring [1]	nº	3.61 ± 0.10[a]	3.76 ± 0.10[b]	3.80 ± 0.05[b]	<0.001
Grower period from 15 to 42 days on trial (39 to 66 days of age)					
Faecal scoring [1]	nº	3.91 ± 0.07[a]	3.95 ± 0.03[ab]	3.98 ± 0.04[b]	0.012
Overall period from 01 to 42 days on trial (25 to 66 days of age)					
Faecal scoring [1]	nº	3.76 ± 0.07[a]	3.86 ± 0.04[b]	3.89 ± 0.03[b]	<0.001

Table 6. Effects of Yeast Cell Wall + Organic Acid blend (YCW+OA) on scoring in post-weaning piglet feces from 25 to 66 days of age (01 to 42 days on trial). [1)] 1: Liquid diarrhea; 2: Pasty feces falling out of shape upon contact with surfaces; 3: Formed feces, soft to cut; 4: Well-formed feces, firm to cut, but not dry; 5: Hard and dry feces.

Following the observed post-weaning diarrhea, the fecal scores were lowest during the first post-weaning weeks. By the fact that these scores were only observed for a few days and the remaining piglets did not exhibit diarrhea, the maximal reduction of the average scores during this critical period was limited to 3.60 in the control group (2nd week after weaning). Because of the reduced incidence rate of post-weaning diarrhea in piglets fed diets containing YCW+OA at both dose levels, means of fecal scoring during the first three weeks after weaning were significantly ($P<0.05$) up to 0.20 scores better than those recorded in the control group. After the first critical post-weaning weeks, a nearer approach to the optimal score of 4.0 (well-formed feces, firm to cut, but not dry) was recorded when using YCW+OA at both dose levels in comparison to the control group. However, responses were not significant ($P>0.05$).

Finally, toxicogenic *E. coli* were the main microbial species detected in fecal samples of piglets with post-weaning diarrhea. Considerable differences between piglets fed diets without or with YCW+OA on the identified prevalence of toxicogenic *E. coli* in fecal samples were not evident.

When the weaning occurs, and the piglets start to receive a solid diet, the digestive physiology changes and several gastro-intestinal disturbances can cause considerable economic losses in the pig industry (SUIRYANRAYNA and RAMANA, 2015). Since the weaning is a complex period where the piglets must deal with several changes, such as the separation from their mother, change in the litter group, and environment, etc. (SUIRYANRAYNA and RAMANA, 2015); all these factors contribute to being a highly stressful period affecting the piglet's health, and negatively impacting on growth. Thus, several feed additives have been studied over the years to help controlling diarrhea and improve the absorption and utilization of the nutrients.

The organic acids can be both bacteriostatic and bactericidal. However, these effects will depend on the levels of their inclusion (SUIRYANRAYNA and RAMANA, 2015). The main action of organic acids in pigs is the reduction of stomach pH (DESAI et al., 2007). In suckling piglets, the acid secretion is low, and the primary source to acidify the gut is the bacterial fermentation of lactose from milk transforming into lactic acid. However, the high level of lactate in the stomach tends to inhibit HCl secretion; that is why the early ingestion of solid feed can reduce the level of lactic acid in the stomach and stimulate the HCl production; but, in practice, creep feed intake is low until 4 weeks of age (PEADAR, et al., 2005)

This scenario contributes to increase the stomach pH and decrease the feed digestion, leading to lower gut bacteria fermentation and colonization, resulting in diarrhea. According to (PARTANEN and MROZ, 1999), the organic acids may influence mucosal morphology, as well as stimulate pancreatic secretions, and they also serve as substrates in intermediary metabolism. Several recent studies have been showing the positive impact of organic acids blend in reducing the bacteria contamination, as well, diarrhea incidence and improving performance in weaned piglets (LEI, et al., 2017; LI, et al., 2018; LONG, et al., 2018; YANG, et al., 2019). These organic acids can be effectively associated with other feed additives, such as prebiotics.

Prebiotics are mostly non-digestible carbohydrates, mainly oligosaccharides, such as fructooligosaccharides (FOS), and mannan oligosaccharides (MOS) (ANDREATTI FILHO and OKAMOTO, 2015). There are also some peptides, lipids, and proteins that can be used as prebiotics. MOS is present in the cell wall of the yeast *Saccharomyces cerevisiae* where are associated with β-glucans. MOS is known for its capacity to agglutinate pathogens that possess *fimbriae,* such as several types of *Salmonella* and *E. coli.* Because these are non-digestible carbohydrates, the "trapped" bacteria will be excreted together with the fecal material (ALCANTARA, et al., 2015; RAHIMI, et al., 2019; HOFACRE, et al., 2017).

The β-glucans can modulate the immune response of the animals because the phagocytic cells can recognize then. Toll-like receptors located on the surface of immunological cells (macrophages, neutrophils, dendritic cells, and natural killer cells) recognize microbial patterns and induce an immediate innate immune response (SHARMA, 2003). After this activation and phagocytosis, the phagocyte presents a processed fragment of the pathogen to the adaptive immune system and stimulates an anti-pathogen response (PETRAVIĆ-TOMINAC, et al., 2010; BONATO, et al., 2020). Therefore, the phagocytes are called antigen-presenting cells. The recognition of pathogens by the innate immune system triggers immediate innate defenses and activation of the adaptive immune response (LEE and IWASAKI, 2007). This response is especially meaningful in animals in reproductive phases, early growth stages, under stress, or in a challenging environment.

The association of both concepts of the yeast cell wall and organic acids blend contemplates different modes of action, and by the results obtained in the present study, are showing to be synergistic in controlling diarrhea incidence, decreasing the number of sick animals by reducing the medicines used (impact in cost) and improving body weight gain.

The study aimed to evaluate the strategic impact of YCW+OA, an anti-bacterial synergistic dry acid mixture (benzoic acid: 19%; sodium benzoate: 15%; formic acid: 25%; phosphoric acid: 4.0%; citric acid: 4.5%; yeast cell wall: 15%) supplemented at the recommended (7 g/kg) or high dose level (14 g/kg) in diets for post-weaning piglets from 25 to 66 days of age housed in a commercial pig breeding farm with a high incidence rate of *Escherichia coli* associated post-weaning diarrheas. It was shown that feeding diets supplemented with YCW+OA even at the low recommended dose level (7 g/kg) resulted in a lower incidence rate of post-weaning diarrhea, lower antibiotic treatments, and reduced mortality rate. Because of the reduced incidence rate of post-weaning diarrhea in piglets fed diets containing YCW+OA at both dose levels, means of fecal scoring in the first three weeks after weaning were significantly better than those recorded in control. Moreover, piglets fed diets containing YCW+OA showed even at the low recommended dose level statistically relevant benefits on the overall body weight gain. However, significant effects on the total feed conversion ratio were limited to the high dose level. These findings suggest that feeding diets supplemented with YCW+OA, even at the recommended low dose level, could provide a lower incidence rate of post-weaning diarrhea. Also, significant benefits on overall body weight gain in post-weaning piglets were observed, from 25 to 66 days of age, under the assumed conditions of circulation of toxicogenic *E. coli* strains, and not identified permanent risk factors. Whereby differences between the low and high dose levels were not significant.

4 | CONCLUSION

The YCW+AO at 7 g/kg or 14 g/kg has significant benefits on overall body weight gain and feed conversion. Besides, provide a lower incidence rate of post-weaning diarrhea in piglets from 25 to 66 days of age under the assumed conditions of *E. coli* strains presence and not identified permanent risk factors, whereby differences between the low and high dose level were not significant.

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ÍNDICE REMISSIVO

A

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