



Respiratory tract clinometry, fat thickness, haematology and productive parameters associated with direct-fed microbials used as growth promoter antibiotic alternative in weaned piglets

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ABSTRACT

The objective was to evaluate the effect of two probiotic yeast strains (*Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004) as a substitute of growth promoter antibiotics on health status and productive parameters in weaned piglets. Commercial line hybrid piglets (Choice n=200), weaned at 21 d age were allotted by sex, and assigned in 4 pens per treatment (2 pens males and 2 pens females), 10 pigs per pen divided into 2 blocks (with or without antibiotics). Dietary treatments included a basal diet (BD) supplemented with probiotic *Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004 (100 g, 1×10^{10} CFU/g, respectively), with or without antibiotics, mixed per ton of growth phases diets. Pigs were fed ad libitum with treatments T1) BD with antibiotics (BD); T2) BD with antibiotics + *Saccharomyces cerevisiae*; T3) BD without antibiotics + *Saccharomyces cerevisiae*; T4) BD with antibiotics + *Kluyveromyces marxianus*; T5) BD without antibiotics + *Kluyveromyces marxianus*. The effects on respiratory tract clinometry, carcass quality, organs weight, blood haematology and productive parameters were evaluated. When clinical signs occurred (diarrhoea, stomach ulcers, respiratory signs), they decreased with both probiotics addition, mainly *Saccharomyces cerevisiae*. The productive parameters promotion by both probiotics was similar than that using antibiotics. The probiotics inclusion increased the carcass weight and significantly reduced the lumbar fat thickness ($P \leq 0.05$). Supplementation with both probiotics demonstrated their ability to substitute the antibiotics use on clinometry, carcass quality and on the productive parameters promotion of weaned piglets.

1. Introduction

Weaning creates stress in piglets causing low and variable feed intake, suboptimal weight gain, diarrhoea episodes and (or) increased morbidity and (or) mortality. The growth after an early weaning has detrimental impacts on growth in the later stages. Pigs that lose weight in the first days after weaning require additional days to reach market weight. Therefore, nutrition and post-weaning management are essential to promote rapid feed intake and reduce mortality and morbidity

(Rhouma et al., 2017).

Antibiotics in pig production have been mainly used for therapeutic and prophylactic purposes. Their administration at sub therapeutic levels stabilizes the gut microbiota and enhance pig growth performance proceeding as antibiotic growth promoters (AGP). They have demonstrated their ability to improve pig growth rate, to reduce morbidity and mortality, and to improve production and reproduction performance (Kazeem et al., 2020). The use of AGP in animals raises concerns related to the selective pressure on bacterial population promoting antibiotic

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resistance (McCrackin et al., 2016; Liu et al., 2018; Lekagul et al., 2019) and, from a consumer standpoint, the need for food safety chemical residues free European Union (EU) prohibited the use of AGP since 2006. (Regulation EC N°1831/2003) and has issued regulations that indicate their replacement.

Among alternatives to AGP there are resources of natural origin that fulfil the same functions, without the risk of the residues presence in meat and/or by-products entails. They include probiotics, prebiotics, symbiotics, organic acidifiers, antioxidants, and plant extracts (Regulation EC N 1831/2000). Probiotics refer to a group of non-pathogenic organisms that, when administered in adequate levels, are known to have beneficial effects on the health of the host animal. At present, probiotics are classified by the US Food and Drug Administration as generally recognized as safe (GRAS) ingredients (FAO/WHO 2002). The most common probiotics are lactic acid-producing bacteria belonging to the genera *Lactobacillus*, *Bifidobacterium*, *Enterococcus* and yeasts such as *Saccharomyces sp.* (Dowarah et al., 2017a; Kiros et al., 2018). Hypothesised mechanisms of action of probiotics are reduction of the pH (unfriendly environment for intestinal pathogens), the adhesion to the intestinal epithelial surface to prevent pathogen attachment. Moreover, the competence for nutrients with pathogens; the production of inhibitory substances (organic acids, hydrogen peroxide, bacteriocins), and the stimulation of specific and nonspecific immunity (IL and IgA) are among other mechanisms (Dowarah et al., 2017b).

Some commercially strains and/or their cell wall components (probiotic-prebiotic) have been reported to improve growth performance, immune function and microbiota composition in weaned piglets (Trckova et al., 2014; Jiang et al., 2015; Kiros et al., 2018). Poloni et al. (2020), showed that *S. cerevisiae* var. *boulaardii* strain included in the diet were effective probiotics in counteracting the toxic effects of harmful aflatoxin B₁ (AFB₁) in livers besides a tendency to improve the histomorphometric parameters and modulating the toxic effect of AFB₁ on intestine. However, they did not demonstrate the influence of this strain on productive parameters when AGP were not present. In addition, there is little information regarding the effects of other yeast species in animals' growth promoting. *K. marxianus* has unique physiological properties such as high growth rate, active metabolic functions, high thermostability and high content of mannans in the cell wall (Lane et al., 2010). Previous studies have demonstrated that *K. marxianus* VM004 (Díaz Vergara et al., 2017) addition might be helpful for animal growth performance and physiological functions. Experiments in ruminants have evidenced that *K. marxianus* addition had the ability to improve feed efficiency and enhance some digestive enzymes activities (Tripathi et al., 2010, 2011). However, to date, the literature has not mentioned the use of probiotic *K. marxianus* to improve production or health promotion in weaning piglets.

Hence, the objective of this study was to evaluate the effect of two probiotic yeast strains (*S. cerevisiae* RC016 and *K. marxianus* VM004) as a substitute of growth promoter antibiotics on productive parameters in weaned piglets under commercial conditions in Argentina. Therefore, health status (respiratory tract clinometry, blood haematology), carcass quality, organs weight, and productive parameters (Total Weight Gain (TWG - Kg), Average Weight Gain (AWG - Kg), Total and daily Feed Intake (DFI - Kg), Feed Conversion ratio (FCR), were evaluated in weaned piglets.

2. Materials and methods

All animal experimentation procedures were approved by the Ethics Committee of the National University of Rio Cuarto in compliance with the regulations of the Subcommittee on Animal Bioethics under the Ethics Committee of Scientific Research, as established in Resolution 253/10 of the Superior Council of the National University of Rio Cuarto. All efforts were made to minimize animal suffering.

2.1. Probiotic microorganisms

Saccharomyces cerevisiae RC016 was isolated from animal ecosystem and identified by molecular techniques through DNA extraction and 18S rRNA and 28S rRNA amplification and analysis, comparing sequences with the Basic Local Alignment Search Tool (BLAST) within the National Centre for Biotechnology Information (NCBI) database (Armando et al., 2012). *Saccharomyces cerevisiae* RC016 was selected based on their probiotic properties, resistance to gastrointestinal conditions, adhesion to intestinal epithelial cells and inhibition of pathogenic bacteria (Armando et al., 2011).

Kluyveromyces marxianus VM004 was isolated from whey and identified by molecular techniques through DNA extraction and Sequencing of ITS regions, comparing sequences with the Basic Local Alignment Search Tool (BLAST) within the National Centre for Biotechnology Information (NCBI) database (Díaz Vergara et al., 2017). *Kluyveromyces marxianus* VM004 was selected based on their probiotic properties, resistance to gastrointestinal conditions, adhesion to intestinal epithelial cells and inhibition of pathogenic bacteria (Díaz Vergara et al., 2017).

2.2. Yeast biomass production and additives formulation

Saccharomyces cerevisiae RC016 and *K. marxianus* CVM004 biomass were obtained from 24 h culture in Yeast-Peptone-Dextrose (YPD) broth in a BioFlo 2000 fermenter (New Brunswick Scientific Co., Inc, Enfield, CT, USA) operated at 28 °C, at 1 x g and 1.5 vvm aeration. The pH value was adjusted to 5 with 6 M NaOH. The working volume was 4 L. The biomass obtained at the end of the fermentation process was centrifuged at 5000 × g at 4°C for 10 minute (min). The concentrated pellet was resuspended in the same volume of cryoprotectant (2.5% maltodextrin), frozen at -80°C and lyophilized. One gram (1 g) lyophilized biomass was hydrated and the viability was confirmed by colony-forming units (CFU/g). Each lyophilized microorganism was considered as a probiotic additive. The colony counts of *S. cerevisiae* RC016 and *K. marxianus* CVM004 in probiotic additive were 1 × 10¹⁰ (CFU)/g, respectively. One hundred grams of *S. cerevisiae* probiotic additive were mixed per ton of the diet corresponding to the different growth phases and 100 g of *K. marxianus* probiotic additive were mixed per ton of the diet corresponding to the different growth phases for the *in vivo* trial.

2.3. Animals and housing

A controlled trial was undertaken (September-December 2019) out in a single-site, farrow-to-finish pig farm, with a breeding herd of one thousand sows, located in Carnerillo city, Córdoba, Argentina. (San José farm), with the following geographical coordinates: Latitude: -32.9233, Longitude: -64.0189 32° 55' 24" south, 64° 1' 8" West. The rainy season lasts for approximately 10 months, with a sliding 31-day rainfall of at least 13 mm and an average total accumulation of 131 mm. The period of the year without rain lasts approximately 2 months with an average total accumulation of 8 mm. The temperate season has an average daily maximum temperature of more than 27°C and an average minimum temperature of 19°C. The cool season has an average daily maximum temperature of less than 18°C and an average maximum of 15°C.

The experiment was carried out in a single-site, farrow-to-finish pig farm, with a breeding herd of one thousand sows. Animals were treated according to standard farm practices unless stated otherwise. Weaned piglets' commercial line hybrids (n=200) (Choice; initial body weight: 6.63 Kg ± 0.47 Kg; weaned at 21 days of age), were randomly selected from the experimental farm. Pigs were allotted according to sex, assigned in 4 pens per treatment (2 pens with males and 2 pens with females), 10 pigs per pen, divided into 2 blocks (100 piglets with antibiotics and 100 piglets without antibiotics). All piglets were identified by a tattoo number according to the treatment, block and individual number. The pigs were housed in pens of 2.6 × 1.8 × 0.9 m³ with woven mesh plastic floor. All pens were equipped with a self-feeder and nipple

drinker to allow ad libitum access to feed and water throughout the 35-day experiment period (21-56 days of age). At 44 days of age the piglets were vaccinated for *Mycoplasma hyopneumoniae* and Circovirus (porcine circovirus type 2 plus *M. hyopneumoniae*). The light was on at 7 am and off at 7 pm daily in the environmental control unit. The room temperature was 25°C – 27°C throughout the experiment.

2.4. Experimental design and dietary treatments

Pigs were fed *ad libitum* with the three (3) regular phase's nutrition schemes of the farm, independently of the treatment. Phase 1 (21 to 32 days of age) diet was administered for 12 days, Phase 2 (33 to 42 days of age) diet was administered for 10 days and Phase 3 (43 to 56 days of age) diet was administered for 14 days. Phase 1 and Phase 2 diets were commercially obtained, while Phase 3 diet was prepared at the farm nutrition plant. They were formulated to meet requirements suggested by the National Research Council [NRC \(2012\)](#) with and without antibiotics, as described in [Table 1](#). Antibiotics were provided during Phase 2 diet (ciprofloxacin 20 g; 1 g/piglet in water during 5 days) and amoxicillin 800 g/Tn feed during 5 days in Phase 3 diet. Sulfachloropyridazine (1000 g/Tn feed) was added the last 3 days of Phase 3 diet. The chemical composition of the diet was estimated by chemical analysis according to [AOAC \(2000\)](#).

Diets were prepared by premixing the probiotic additives separately and then mixing with the different diet phases in an industrial mixer. Diets were sampled for AFB₁ analysis following the recommendations of the European Union ([Regulation 401/2006](#) and its modification by [Regulation 178/2010](#)). The AFB₁ concentration of each diet was determined by HPLC fluorescence detector according to [Trucksess et al. \(1994\)](#). The analysis was developed in triplicates (results in [table 1](#)).

Dietary treatments included a basal diet supplemented with probiotic additives *S. cerevisiae* RC016 and *K. marxianus* VM004 (100 g, 1 × 10¹⁰ CFU/g, respectively), with or without antibiotics, and mixed per ton of the different growth phases diets. Pigs were fed *ad libitum* with the following treatments T1) basal diet with antibiotics (BD); T2) BD with antibiotics + *S. cerevisiae*; T3) BD without antibiotics + *S. cerevisiae*; T4) BD with antibiotics + *K. marxianus*; T5) BD without antibiotics + *K. marxianus*.

2.5. Experimental procedures, and sampling

Individual weaning piglets weight were recorded at the beginning of the experiment and at the end of every phase diet. The amount of feed offered to each pen was recorded at the beginning of each diet phase; the amount of feed left in the feeder was weighted and the difference was calculated to estimate total and daily feed intake. The Total Weight Gain (TWG - Kg), Daily Weight Gain (DWG - Kg), Total and Daily Feed Intake (DFI - Kg), and Feed Conversion Ratio (FCR), (Daily Feed Intake: Daily Weight Gain) were determined for each treatment and for sex at 33, 43 and 56 days of age.

At the end of the feeding trial (56 day of age, 35 days of assay), all pigs were sacrificed by an intravenous injection of sodium pentobarbital (50 mg/Kg body weight), to evaluate carcass quality, blood haematology, macroscopic lesions in nasal turbinates, lungs, stomach, liver, spleen and kidneys. Also, the absolute and relative weights of liver, lung, kidney, and spleen were determined.

2.6. Carcass quality determination

The carcass weight (Kg) was recorded with head and shanks. Carcass length (cm) was measured from the middle of the anterior edge of the first rib to the anterior edge of the aitch bone or pubis according to [Bereskin and Steele \(1988\)](#).

Back-fat thickness (mm) was evaluated at the level of the first, tenth and last rib by measuring subcutaneous fat over the middle of *Longissimus thoracis* and *lumborum* muscle with a Vernier caliper and

Table 1
Composition of the basal diet for the three phases, as fed-basis

Item	Unit	Growing Stage		
		Phase 1(21 to 32 days of age)	Phase 2(33 to 42 days of age)	Phase 3(43 to 56 days of age)
Ingredients				
Corn (ground)	Kg	470.5	493.5	560.5
Soybean meal (47% CP)	Kg	255	368	350
Soybean oil	Kg	24	36	37
Dextrose plus	Kg	0.5	0.5	0.5
Starter concentrate ^a	Kg	250		
Growing concentrate (NF4) ^a	Kg		100	
Growing concentrate (NF5) ^a	Kg			50
Calculated nutrient content ^b				
Dry Matter	%	92.0074	90.6097	88.8411
C P	%	22.7043	22.2336	19.8668
ME	Kcal	3,676.6047	3,595.3111	3,429.3360
Total Lysine	%	1.6645	1.6005	1.4165
Dig Lysine	%	1.5500	1.5000	1.3123
Dig Methionine	%	0.6105	0.6122	0.5481
Dig Cysteine	%	0.3281	0.2928	0.2500
Dig Met + Cyst	%	0.9300	0.9000	0.8771
Dig Tryptophan	%	0.3565	0.3450	0.2429
Dig Threonine	%	1.0075	0.9750	0.8373
Dig Arginine	%	1.1428	1.1900	
Dig Valine	%	0.6391	0.7395	
Crude fat	%	9.1526	8.3909	4.9587
Crude fibre	%	1.9073	2.3984	3.4877
Calcium	%	0.8575	0.8500	0.6679
Total Phosphorus	%	0.5934	0.5710	0.6504
Available Phosphorus	%	0.5663	0.5046	0.4362
Lactose	%	15.0000	7.5000	
Linoleic Acid (C18:2)	%	2.7068	3.0688	2.4500
Choline	µg/g	735.0000	735.0000	593.2238
Zinc	µg/g	3,000.0000	3,000.0000	1,639.1250
Copper	µg/g	266.2000	266.2000	263.5000
Selenium	µg/g	0.4050	0.4050	0.3375
Iron	µg/g	90.3900	90.3900	75.4750
Sodium	%	0.4605	0.3402	0.2196
Chlorine	%	0.5061	0.3733	0.3000
Ash	%	5.9760	5.7318	4.6212
Aflatoxin B ₁	ng/g	27.61	20.97	310.07

CP=Crude Protein; ME=metabolizable energy.

^a Provided the following per kilogram of diet: 12,000 IU vitamin A as vitamin A acetate; 2,500 IU vitamin D3; 30 IU vitamin E as DL- α -tocopheryl acetate; 12 µg vitamin B12; 3 mg vitamin K as menadione sodium bisulphate; 15 mg D-pantothenic acid as calcium pantothenate; 40 mg nicotinic acid; 400 mg choline as choline chloride; 30 mg Mn as manganese oxide, 80 mg Zn as zinc oxide, 90 mg Fe as iron sulphate, 10 mg Cu as copper sulphate; 0.35 mg as ethylenediamine dihydroiodide and 0.3 mg Se: as sodium selenite.

^b Based on [NRC \(2012\)](#) values.

expressed according to [Ziegler \(1968\)](#). All of these parameters were measured individually (per animal) in each experimental group.

2.7. Pigs health status. Clinometric and macroscopic lesions scores

Pigs were evaluated clinically every day to evaluate the occurrence

of cough, sneezing and diarrhoea. Presence or absence of diarrhoea (liquid faeces on the floor and/or dirty anal region) was determined. At the end of the experiment, the percentage of days with diarrhoea occurrence was calculated for the experimental period according to Dos Anjos et al. (2019). Additionally, nasal turbinate, lungs and stomach lesions were scored according to Donko et al. (2005), Hessing et al. (1992) and Morrison et al. (1985), respectively.

2.8. Absolute and relative organs weight

The liver, lung, kidney, and spleen were obtained immediately after evisceration for absolute and relative weights (g) determinations. The relative organ weight was calculated as follows:

$$\text{Relative organ weight (\%)} = \text{Weight of organ (g)} / \text{Body weight (g)} \times 100$$

2.9. Haematological analysis

Blood samples (5 mL) were collected at 32 days of age via the superior vena cava into vacuum blood collection tubes with heparin. These same selected pigs were then part of the necropsied animals.

Samples were immediately transported to the laboratory and analysed for haematological profile. The samples were centrifuged at 4°C for 5 min (3,000 × g) and stored at 4°C until analysis. The erythrocyte histogram and leukogram were obtained by an automated analysis process (Diatron MI PLC, Hungary). The haematological parameters such as erythrocytes (Er millions/ μ L), haemoglobin (Hgb g/dL), haematocrit (Ht %), mean corpuscular volume (MCV fL), mean corpuscular haemoglobin (MCH pg), mean corpuscular haemoglobin concentration (MCHC %), total proteins (TP gr/dL), fibrinogen (Fib mg/dL), leukocytes (LEK miles/ μ L), segmented neutrophils (Neut miles/ μ L), segmented neutrophils percentage concentration (Neut %), lymphocytes (Lymph miles/ μ L), lymphocytes percentage (Lymph %), monocytes (Mono miles/ μ L), monocytes percentage (Mono %), eosinophils (Eos miles/ μ L), eosinophils percentage (Eos %) and platelets (Plt miles/ μ L) were determined and quantified.

2.10. Statistical analyses

Daily and Total Weight Gain were determined using each animal as an experimental unit. Total and Daily Feed Intake and carcass quality were determined using each pen as an experimental unit. The data was analysed by general linear and mixed model (GLMM) using Statistical Analysis System software (InfoStat 2012. University of Cordoba, Argentina). ANOVA was run; means and standard deviation of data were compared using the Fisher's protected least significant test (LSD) ($P < 0.05$).

3. Results

3.1. Aflatoxin B₁ determination in diets

The presence of AFB₁ was detected in diets of all phases as follows: Phase 1 had 27.61 ng/g, Phase 2 had 20.97 ng/g and Phase 3 had 310.07 ng/g (Table 1).

3.2. Productive parameters and carcass quality

Table 2 shows the effect of *S. cerevisiae* RC016 and *K. marxianus* VM004 probiotic supplementation on productive parameters at the end of the experimental period (56 days of age and 35 days of assay). Treatments with probiotics (with and without antibiotics) were compared with control treatment and results showed no statistical differences among them ($P \geq 0.05$), the productive parameters promotion by both probiotics was similar than that using antibiotics. Although not statistically significant, male pigs had lower FC ratios than those from females ($P \geq 0.05$).

Table 3 shows the effect of *S. cerevisiae* RC016 and *K. marxianus* VM004 probiotic supplementation on carcass quality at the end of the

Table 2

Effect of *Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004 probiotic supplementation on productive parameters at the end of the experimental period

T [†]	A	Sex	Productive parameters			
			TWG (Kg)(mean \pm SD)	DWG (Kg) (mean \pm SD)	DFI (Kg) (mean \pm SD)	FCR
1	+	F	12.11 \pm 1.11	0.346 \pm 0.02	0.41 \pm 0.05	1.51
		M	13.17 \pm 2.14	0.376 \pm 0.04	0.43 \pm 0.04	1.46
2	+	F	8.97 \pm 1.53	0.256 \pm 0.03	0.39 \pm 0.04	1.97
		M	11.73 \pm 1.37	0.335 \pm 0.02	0.41 \pm 0.04	1.56
3	-	F	10.31 \pm 2.27	0.295 \pm 0.04	0.41 \pm 0.05	1.81
		M	10.01 \pm 1.70	0.286 \pm 0.03	0.38 \pm 0.04	1.71
4	+	F	11.69 \pm 1.99	0.334 \pm 0.04	0.48 \pm 0.05	1.87
		M	11.15 \pm 1.81	0.319 \pm 0.03	0.34 \pm 0.03	1.37
5	-	F	10.60 \pm 2.04	0.303 \pm 0.04	0.37 \pm 0.03	1.59
		M	11.68 \pm 2.39	0.334 \pm 0.04	0.41 \pm 0.05	1.59

A= Antibiotics (+): with antibiotics, (-): without antibiotics.

F: female, M: male,

Total Weight Gain (TWG), Daily Weight Gain (DWG), Daily Feed Intake (DFI), Feed Conversion Ratio (FCR).

N= 200 pigs, (100 Females,100 Male). Each mean based on 4 replicates with 10 pigs per replicate, 20 males and 20 females per treatment.

There were no significant differences according to the Fisher's protected least significant difference Test ($P \geq 0.05$).

[†] Dietary treatments included a basal diet supplemented with probiotic additives *S. cerevisiae* RC016 and *K. marxianus* VM004 (100 g, 1×10^{10} CFU/g, respectively), with or without antibiotics, and mixed per ton of the different growth phases diets. Pigs were fed ad libitum with the following treatments T1) basal diet with antibiotics (BD); T 2) BD with antibiotics + *S. cerevisiae*; T3) BD without antibiotics + *S. cerevisiae*; T4) BD with antibiotics + *K. marxianus*; T5) BD without antibiotics + *K. marxianus*.

Table 3

Effect of *Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004 probiotic supplementation on carcass quality at the end of the experimental period

T [†]	A	Carcass quality [‡] (mean \pm SD)				
		Carcass weight(Kg)	Carcass length(cm)	Fat thickness (mm)		
				Dorsal	Lumbar	Cervical
1	+	13.28 \pm 1.02	50.5 \pm 9.6	11.5 \pm 2.1	10 \pm 2.1 ^c	11.5 \pm 0.7 ^{ab}
				2.1		
2	+	14.12 \pm 0.85	52.0 \pm 2.8	10.5 \pm 1.5	4.2 \pm 0.9 ^a	10.7 \pm 1.7 ^{ab}
				1.5		
3	-	14.37 \pm 0.68	55.5 \pm 2.1	6.4 \pm 1.2	4.7 \pm 0.9 ^a	12.0 \pm 1.2 ^{ab}
				1.2		
4	+	13.40 \pm 0.45	55.0 \pm 1.4	9.5 \pm 1.5	7.7 \pm 1.1 ^b	12.5 \pm 1.1 ^b
				1.5		
5	-	12.21 \pm 0.21	52.2 \pm 3.5	7.2 \pm 1.2	6.5 \pm 1.0 ^b	8.7 \pm 1.2 ^a
				1.2		

a,b Values that differ significantly ($P < 0.05$) according to the Fisher's protected least significant difference Test.

A= antibiotics (+): with antibiotics, (-): without antibiotics.

[†] Dietary treatments included a basal diet supplemented with probiotic additives *S. cerevisiae* RC016 and *K. marxianus* VM004 (100 g, 1×10^{10} CFU/g, respectively), with or without antibiotics, and mixed per ton of the different growth phases diets. Pigs were fed ad libitum with the following treatments T1) basal diet with antibiotics (BD); T 2) BD with antibiotics + *S. cerevisiae*; T3) BD without antibiotics + *S. cerevisiae*; T4) BD with antibiotics + *K. marxianus*; T5) BD without antibiotics + *K. marxianus*.

[‡] N= 200 pigs, (100 Females,100 Male). Each mean based on 4 replicates with 10 pigs per replicate, 20 males and 20 females per treatment.

experimental period. Recorded values of carcass length and weight did not show differences among treatments nor sex ($P \geq 0.05$). The inclusion of the probiotic additives showed that *S. cerevisiae* RC016 obtained the highest carcass values followed by *K. marxianus* VM004 treatments.

The lumbar fat thickness was significantly reduced with the inclusion of both probiotics ($P < 0.05$), compared to the control treatment.

S. cerevisiae RC016 showed the lowest values of lumbar fat thickness followed by *K. marxianus* VM004 treatments. However, the dorsal and cervical fat thickness did not show significant differences with the addition of *S. cerevisiae* RC016 ($P \geq 0.05$), but showed a tendency to reduce them. The inclusion of the probiotic *K. marxianus* VM004 did not show significant differences compared to the control treatment ($P \geq 0.05$), on dorsal and cervical fat thickness.

3.3. Clinometric and macroscopic lesions scores

Table 4 shows clinical signs and macroscopic lesion scores of the different treatments. Signs of diarrhoea were observed only during the first week of the trial just after weaning. Piglets that received *S. cerevisiae* RC016 probiotic additive had the least clinical signs of diarrhoea; although the highest percentage of diarrhoea was observed in animals with *K. marxianus* VM004 compared to the control group, the average time of duration of diarrhoea of control group was 5 days, while in the probiotic treatments it lasted 2 to 3 days. Stomach ulcer index and respiratory signs decreased with the addition of both probiotics, separately. Control treatment animals had the highest (10%) percentage of coughs and sneezing. A low (5%) percentage of cough and sneezing was observed in the animals with *K. marxianus* VM004, followed by the animals that received *S. cerevisiae* RC016.

In all treatments, the highest percentage of lung lesions was observed in the right and left middle lobes. Animals in the control group had the highest percentages of pneumonia, followed by the animals fed *K. marxianus* VM004, while the lowest percentages of pneumonia were observed in animals fed *S. cerevisiae* RC016. The nasal turbinate atrophy index showed the same behaviour as the pneumonia percentages.

3.4. Absolute and relative organs weight

No significant differences ($P \geq 0.05$) in the absolute organs weights (liver, lung, kidney and spleen) of pigs that received the different treatments were observed. However, significant differences were observed in the relative organ weights ($P < 0.05$). With the inclusion of *S. cerevisiae* RC016 without antibiotics, the relative liver and lung weight showed significant differences compared to the control ($P < 0.05$). While, the inclusion of *K. marxianus* VM004 without antibiotics, showed significant differences in relative lung weight compared to the control ($P < 0.05$) (data not shown) (Table 1 – supplementary material).

Table 4

Effect of *Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004 probiotics supplementation on clinical signs and macroscopic lesions scores at the end of the experimental period

T [†]	A	Macroscopic Lesions Scores											Clinical signs (% of pigs)		
		Lung pneumonia (%) [‡]						Nasal turbinate atrophy index [‡]					Stomach ulcer index [‡]	Diarrhoea [‡]	Respiratory [‡]
		LA	RA	LM	RM	I	LC	RC	LD	LV	RD	RV			
1	+	2	7	20	20	2	6	5	0	2	0	1	2	10	30
2	+	0	0	5	3	0	2	0	0	0	0	0	2	0	10
3	-	0	0	0	2	0	0	0	1	2	1	1	1	20	10
4	+	0	0	22	8	2	5	2	0	1	0	1	1	30	10
5	-	0	0	7	2	0	0	2	0	1	0	1	2	20	0

There were no significant differences according to the Fisher's protected least significant difference Test ($P \geq 0.05$).

+ *K. marxianus*; T5) BD without antibiotics + *K. marxianus*.

Lung lesions (by lung lobe): left anterior lobe (LA); right anterior lobe (RA); left middle lobe (LM); right middle lobe (RM); intermediate lobe (I); left caudal lobe (LC); right caudal lobe (RD). Nasal turbinate (by turbinate): left dorsal turbinate (LD); left ventral turbinate (LV); right dorsal turbinate (RD); right ventral turbinate (RV). A= Antibiotic (+): with antibiotics, (-): without antibiotics.

[†] Dietary treatments included a basal diet supplemented with probiotic additives *S. cerevisiae* RC016 and *K. marxianus* VM004 (100 g, 1×10^{10} CFU/g, respectively), with or without antibiotics, and mixed per ton of the different growth phases diets. Pigs were fed *ad libitum* with the following treatments T1) basal diet with antibiotics (BD); T 2) BD with antibiotics + *S. cerevisiae*; T3) BD without antibiotics + *S. cerevisiae*; T4) BD with antibiotics [‡] N= 200 pigs.

3.5. Haematological parameters

There were no significant differences with the supplementation of *S. cerevisiae* RC016 and *K. marxianus* VM004 probiotic additives with or without antibiotics compared to the control treatment ($P \geq 0.05$) when haematological profile of pigs under different treatments were compared (Table 2 supplementary material).

4. Discussion

The effects of sub-therapeutic levels of AGP in pig diets have generated increased interest in the pigs' production. Antibiotic application for growth promotion have drawn pressing public health concerns, due a problem such as antibiotic resistance and antibiotic residues caused by the abuse of antibiotics have been frequently reported worldwide (Li et al., 2020). Their elimination could increase disease problems and reduce growth performance. Therefore, it is necessary to make changes in both management and nutrition.

This paper studied the efficiency of two probiotic yeasts, *S. cerevisiae* RC016 and *K. marxianus* VM004 as growth promoter antibiotics usage alternative on respiratory tract, clinometry, fat thickness, haematology and productive parameters in weaned piglets under commercial conditions of Argentina.

In agreement with our results, Dávila-Ramírez et al. (2020) reported an improvement in ADWG and FC ratio when *S. cerevisiae* (N. Strain 7907) (0.2-0.3%) was added to weanling pigs feed. Also, Wang et al., (2017) showed that the ADWG of piglets fed *B. amyloliquefaciens* (2×10^8 CFU/g) was significantly improved compared to the antibiotic group. Here, carcass quality studies demonstrated the ability of probiotics to replace antibiotics, but also to improve this parameter by reducing the dorsal and lumbar fat thickness. Similar results were obtained by GaneshKumar et al. (2009) that indicated an improvement in carcass weight and a decrease in fat thickness by the supplementation of a commercial probiotic without antibiotics (5 g/pig/day).

In the present study, the supplementation with probiotics did not affect the organs weight. Different authors using mannan oligosaccharide (MOS) demonstrated the effect of its inclusion in piglet diets. Our results are agreement with Sant'ana et al. (2017) observed no influence of MOS on organs weight, whereas, Dos Anjos et al. (2019) observed an incensement of spleen weight with the addition of β -glucans, glucmannans, and MOS piglet diets. On the other hand, in the present work, the blood metabolite levels were maintained with the addition of yeasts. Similarly, Dávila-Ramírez et al. (2020) demonstrated that levels of leukocytes, lymphocytes, monocytes, erythrocytes, haemoglobin,

haematocrit, mean corpuscular volume, MCHC, and AED did not exhibit changes with *S. cerevisiae* N. 7907 supplementation. In contrast, Czech et al. (2018) showed high values of red line cells with the inclusion of *Yarrowia lipolytica* and *S. cerevisiae*. Similarly, Van der Peet-Schwering et al. (2007) demonstrated great values of red line cells with the inclusion of 0.125% of yeast culture (commercial) without antibiotics in weaning pigs.

The *E. coli* is the main cause of diarrhoea in post weaning pigs and several studies have shown the beneficial effect of probiotics supplementation to reduce its incidence. In the present study the incidence of diarrhoea was reduced in the animals supplemented with probiotics. These results agree with Campbell et al. (2013) and Trckova et al. (2014) who showed the reduction of diarrhoea after probiotics supplementation. Similarly, Dowarah et al. (2017b) reported a reduction of diarrhoea by the supplementation of probiotic *L. acidophilus* and *P. acidilactici*. Also, Hancox et al. (2015) reported a reduction of diarrhoea by the dietary supplementation of *S. cerevisiae* var. *boulardii* (3.3×10^9 CFU). Probiotics could improve the overall health of animals by preventing gut microbiota imbalance and therefore, improving gut health via modifying the gut microbial population.

In the present work, the administration of probiotic yeasts in the pigs' diet decreased the incidence of lung lesions. In agreement with the present results Ayala et al. (2012) showed that the addition of *Bacillus subtilis* for 5 weeks diminished the risks of respiratory lesions affecting the lungs in pigs. Also, Wang et al. (2018) showed a predominance of *Lactobacillus* in the healthy pig groups, demonstrating the protective effect of *Lactobacillus* against respiratory diseases. Recent research has shown that changes in intestinal microbial composition and function could induce alterations in the respiratory mucosa, which can lead to the development of diseases in the lungs (Surendran Nair et al., 2019). Other studies have demonstrated the role of the gut microbiota in swine respiratory infections, particularly porcine reproductive and respiratory syndrome (PRRS), porcine circovirus type 2 (PCV2) and *M. hyopneumoniae* (Siqueira et al., 2017; Niederwerder, 2017). However, the mechanisms by which the gut microbiota could affect the physiology and pathology of the lungs are not yet known Mortaz et al. (2013). explained that the beneficial effects of probiotics are based on their ability to differentially regulate the production of anti and pro inflammatory immune responses.

5. Conclusions

The administration of two probiotic yeast strains (*Saccharomyces cerevisiae* RC016 and *Kluyveromyces marxianus* VM004) without antibiotics addition in pigs post-weaning did not affect health and growth performance of piglets in the present conditions. The dietary inclusion of the two probiotic yeast strains improved some of the health parameters of post-weaning pigs reared under commercial conditions of Argentina. This study revealed that these yeasts could be a feasible alternative to the replacement of antibiotics as growth promoters obtaining by-products without antibiotic residues.

Animal welfare statement

The working protocol and the used techniques were approved and comply with the regulations of the Subcommittee on Animal Bioethics under the Ethics Committee of Scientific Research, as established in Resolution 253/10 of the Superior Council of the National University of Rio Cuarto. All efforts were made to minimize animal suffering

Ethical Statement

Lilia Renee Cavaglieri reports financial support was provided by National University of Rio Cuarto School of Exact Physical Chemistry and Natural Sciences. Lilia Cavaglieri reports a relationship with Professor and researcher that includes: employment. Universidad Nacional

de Rio Cuarto has patent pending to Licensee. There are no conflicts of interests

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2022.100246.

Appendix A

Supplementary data

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