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Beneficial effects of *Saccharomyces cerevisiae* RC016 in weaned piglets: *in vivo* and *ex vivo* analysis

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RESEARCH ARTICLE

Abstract

Probiotics represents an alternative to replace antibiotics as growth promoters in animal feed and are able to control enteric bacterial diseases and to improve gut immunity. *Saccharomyces cerevisiae* RC016 showed previously inhibition/coagregation of pathogens) and mycotoxins adsorbent ability (aflatoxin B_1, ochratoxin A and zearalenone). The aim of this work was to evaluate beneficial properties of *S. cerevisiae* RC016 in a non-inflammatory *in vivo* model in weaned piglets and in an intestinal inflammation *ex vivo* model induced by the mycotoxin deoxynivalenol (DON). Secretory immunoglobulin A (s-IgA) levels, intestinal cytokines, goblet cells and production parameters were evaluated in a pig model. For the *in vivo* assays, twelve pigs were weaned at 21 days and assigned to two groups: Control (n=6) and Yeast (n=6). Animals received yeast strain for three weeks. After 22 days the small intestine was recovered for determination of goblet cells and s-IgA. For the *ex vivo* assay, jejunal explants were obtained from 5 weeks old crossbred piglets and treated as follow: (1) control; (2) treated for 3 h with 10 µM DON used as an inflammatory stressor; (3) incubated with 10^7 cfu/ml yeast strain; (4) pre-incubated 1 h with 10^7 cfu/ml yeast strain and then treated for 3 h with 10 µM DON. CCL20, interleukin (IL)-1β, IL-8 and IL-22 gene expression was determined by qPCR. Oral administration of *S. cerevisiae* RC016 increased s-IgA, the number of goblet cells in small intestine and all the growth parameters measured. In the *ex vivo* model, the cytokine profile studied showed a potential anti-inflammatory effect of the administration of the yeast. In conclusion, *S. cerevisiae* RC016 is a promising candidate for feed additives formulation to improve animal growth and gut immune system. This yeast strain could be able to improve the gut health through counteracting the weaning-associated intestinal inflammation in piglets.

Keywords: probiotic yeast, pigs, gut ecosystem, growth performance, feed additives

1. Introduction

Weaning is one of the most stressful events in the pig's life that contributes to intestinal and immune system dysfunctions. The period following weaning, characterised by sub-optimal growth, deteriorated feed efficiency and changes in the gastrointestinal ecosystem, can induce diarrhoea (Lallès *et al.*, 2007; Pluske *et al.*, 2013; Rist *et al.*, 2013). Also, in this period there are increases in the expression of inflammatory cytokines in the intestine of piglets that can lead changes on gut integrity (Pié *et al.*, 2004).

It is important to point out here that piglets can reach high performance levels with optimum feeding strategies. Subtherapeutic use of antibiotics has widely been applied to solve post-weaning problems (Barton, 2000) resulting in the development of antibiotic resistance. In 2006, the European Union banned antibiotics as growth promoters in animal feed. Consequently, there is great interest in studying alternatives to reduce/eliminate the use of antibiotics in

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feeds, including the administration of probiotics, which are defined as live microorganisms that confer a health benefit to the host (FAO, 2001).

In piglets, the intestine plays significant digestive and immunological functions. During the postnatal period, the intestine is in contact with large amounts of new antigens. The function of the intestine during post weaning is often evaluated based on changes in the structure of mucosa and the degree of intestinal integrity. Also, these aspects affect nutrient digestibility, feed efficiency, resistance to diseases and animal performance (Barszcz and Skomiał, 2011).

Previous work demonstrated that the strain *Saccharomyces cerevisiae* RC016 isolated from the gut of a healthy pig is able to survive under gastrointestinal conditions and has beneficial properties such as aggregation/inhibition of pathogenic bacteria, ability to improve ruminal fermentation and the capacity to bind mycotoxins under gastrointestinal conditions (Armando *et al.*, 2011, 2012a,b, 2013; Dogi *et al.*, 2011). *In vivo* assays in a murine model, demonstrated that *S. cerevisiae* RC016 was able to modulate the gut immune system and the associated microbiota by increasing the number of immunoglobulin A (IgA)+ cells, decreasing tumour necrosis factor (TNF)-α levels in the small intestine with increase of interleukin (IL)-10/TNF-α ratio and by decreasing the enterobacteria counts in the caecum (García *et al.*, 2016). Furthermore, this yeast strain did not induce genotoxicity or cytotoxicity in rats (González Pereyra *et al.*, 2014).

Among the proposed alternatives to antibiotics, probiotics are considered good candidates since they can improve intestinal health by stimulating the immune system and modulating gut microbiota (De Moreno de LeBlanc *et al.*, 2008; Dogi *et al.*, 2008).

There are different mechanisms to experimentally simulate the inflammation that occurs naturally in animals as result of weaning or the processes associated with weaning (such as colitis). There are inflammation-inducing chemical agents, such as trinitrobenzene sulfonic acid and biological agents, such as the mycotoxin deoxynivalenol (DON).

After weaning, pigs are subjected to a conventional balanced diet, where mycotoxins are present as natural contaminants of grains, seeds and forages, which are the main raw materials used in the preparation of foods for animal nutrition. DON is a trichothecene mycotoxin, produced mainly by *Fusarium graminearum*, and it is one of the most frequently found mycotoxin in small cereal grains (Bando *et al.*, 2007; Freire *et al.*, 2007). Exposure to DON can induce gastrointestinal inflammation and necrosis within the intestinal tract and disturbs the gut barrier function (Pinton *et al.*, 2010; Ghareeb *et al.*, 2015).

According to the above, we hypothesised that *S. cerevisiae* RC016 administration could improve intestinal health leading to better general health and that this yeast strain could be able to reduce intestinal inflammation. Therefore, the aim of this work was to evaluate beneficial properties of *S. cerevisiae* RC016 in a non-inflammatory *in vivo* model in weaned piglets and in a model of intestinal inflammation *ex vivo* induced by the mycotoxin DON.

2. Materials and methods

In vivo experimental design

Animals and diets

The assay was carried out according to international health standards and ethical guidelines and the experimental protocol comply with the regulations of the Research Ethics Committee, as established in Resolution 253/10 of the Superior Council of the National University of Rio Cuarto. The animals maintained good health throughout the experimental period.

A total of twelve pigs (hybrid race between 5,500 and 6,100 kg) were weaned at 21 d of age, and allowed to acclimate for 7 d. After the acclimatisation period, pigs were randomly assigned to individual pens (1.22×0.61 m). Litters were divided equally between both treatments and fed during 22 days. During this period, pigs had *ad libitum* access to water and feed supplemented (n=6) or not (n=6) with *S. cerevisiae* RC016 (1×10^7 cfu/g of feed). The experiment was repeated twice. The animals were not fed commercial feed but were fed a diet based on ground maize without any additive or antibiotics. Its chemical composition is described in Table 1. *S. cerevisiae* RC016 was produced using yeast peptone dextrose as culture medium in a bio-reactor (BioFlo 2000, New Brunswick Scientific, Edison, NJ, USA) at 28 °C, 3.62232 *g* and 1.5 vvm (l/min) for 24 h. After that, the culture was centrifuged at 5,000×*g* for 20 min. The pellet was harvested and cell viability was tested by trypan blue exclusion assay. The general health status of pigs was evaluated by recording any changes in behaviour, activity, posture, feed and water intake, possible illness and deaths. Animals were weighed every 3 d. The feed given

Table 1. Physico-chemical composition of experimental pigs' diet.

Ingredient	Percentage (%)
Protein	7.1
Raw fibre	3.5
Ash	1.8
Humidity	12.1
Total fat	11.3

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and the remainder were weighed daily to estimate the daily feed intake (DI). After 22 d of feeding period, animals were sacrificed by a lethal injection of sodium pentobarbital.

Histomorphometry, secretory IgA and microbiota from intestine

Portions of duodenum approximately 6 mm^2 tissue samples were fixed in 4% (v/v) buffered-saline formaldehyde pH 7.2-7.4 at 4 °C, dehydrated in a graded series of ethanol (30, 50, 70, 80, 90, 95 and 100%) and xylene solutions, embedded in paraffin and cut in ±4 µm histological serial-sections. The histological sections were stained with haematoxylin/eosin for microscopic analysis. Morphometric measurements of intestinal variables were carried out on two slides per animal/intestine, two sections per slide and five fields per section. The morphometric measurements taken from the intestinal histological sections included villus length and width, intestinal crypt depth and quantification of goblet cells. Digital images were captured with an Axiophot microscope (Carl Zeiss, Thornwood, NY, USA) fitted with high-resolution Powershot G6 7.1 megapixels digital camera (Canon Inc., Tokyo, Japan). Digital image analysis and morphometric measurements were performed with Axiovision AxioVs40 V4.6.3.0. software (Carl Zeiss, Göttingen, Germany).

Intestinal contents were collected from the small intestines of pigs with 500 µl of cold phosphate buffer saline solution, maintained on ice and then centrifuged at 5,000×*g* for 15 min at 4 °C. The supernatants were recovered and stored at -20 °C until secretory IgA (s-IgA) determination by ELISA (Bethyl, Montgomery, TX, USA).

The caecum from pigs were aseptically removed, weighed and placed in sterile bottles containing 50 ml of 0.1% peptone solution. The samples were homogenised immediately under sterile conditions. Serial dilutions of the homogenised samples were performed and aliquots (0.1 ml) of dilutions were spread onto the surface of the following agarised media obtained from Britania (Buenos Aires, Argentina): De Mann-Rogosa-Sharp (MRS) for total lactobacilli, and MacConkey for total enterobacteria. Plates were aerobically incubated at 37 °C for 24-48 h.

Performance parameters

Growth performance was determined as total weight gain (TWG), calculated as the difference between the weight of animals in the beginning and at the end of the experiment, and progressive weight gain (PWG) calculated considering the progressive weight gain of animals (weighed 3 times a week) during the experiment. Feed efficiency (FE) was calculated as TWG/DI and feed conversion rate (FCR) as DI/TWG. The FCR is a measure of the amount of feed required to produce 1 kg of pig live weight. Carcass weight and carcass performance were evaluated as the body of the slaughtered animals after removal of the offal (viscera, skin and hooves). All of these growth parameters were measured individually (per animal) and per experimental group and statistically analysed, *P*-values <0.05 are considered significant.

***Ex vivo* experimental design**

Toxins

Purified DON was purchased from Sigma-Aldrich (Saint Louis, MO, USA). This toxin was dissolved in water and stored at -20 °C until use.

Intestinal jejunal explants

Jejunal explants were obtained from 5 weeks old crossbred castrated male piglets as described previously (Lucioli *et al.*, 2013; Pierron *et al.*, 2016a). All animal experiments were performed at Toxalim animal facility under the guidelines of the French Minister of Agriculture (agreement APAFiS#6303_2016080314392462). Two authors (I.P.O., D.P) have an official agreement with the French Veterinary Services permitting animal experimentation. Explants were treated: (1) with complete William's Medium E (Sigma, Saint Quentin Fallavier, France), control group; (2) exposed to 10^7 cfu/ml *S. cerevisiae* RC016 in complete medium; (3) pre-treated with complete medium 1 h and then exposed to 10 µM DON in complete medium; or (4) pre-incubated 1 h with 10^7 cfu/ml *S. cerevisiae* RC016 and then exposed for 3 h to 10 µM DON in complete medium. The explants were incubated at 39 °C.

Gene expression analysis by RT-qPCR.

For the gene expression analysis, jejunal explants were treated for 4 h and stored at -80 °C before RNA extraction. Total RNAs were extracted in lysing matrix D tubes (MP Biomedicals, Illkirch, France) containing guanidine thiocyanate-acid phenol (Eurobio, Courtaboeuf, France). The quantity of these RNAs was assessed (Nanodrop, ThermoFisher, Waltham, MA, USA). Reverse transcription and RT-qPCR steps were performed as already described (Alassane-Kpembi *et al.*, 2017; Pierron *et al.*, 2016b). Primers are indicated in Table 2. Amplification efficiency and initial fluorescence were determined by the ΔCt method. Obtained values were normalised using two reference genes, ribosomal protein L32 (RPL32) and cyclophilin A. Expression levels of mRNA were expressed relative to the mean of the control.

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Table 2. Primer sequences used for RT-qPCR analysis.

Gene symbol	Gene name	Primer sequence[1]	References
Cyclo A	Cyclophilin A	F: CCCACCGTCTTCTTCGACAT	NM_214353
		R:TCTGCTGTCTTTGGAACTTTGTCT	Cano *et al.*, 2013
RPL32	Ribosomal protein L32	F: AGTTCATCCGGACCAGTCA	NM_001001636
		R: GAACCTTCTCCGCACCCTGT	Alasanne_Kpembi *et al.*, 2017
IL22	Interleukin-22	F: AAGCAGGTCCTGAACTTCAC	AY937228
		R: CACCCTTAATACGGCATTGG	Cano *et al.*, 2013
CCL20/MIP3α	C-C chemokine ligand 20	F: GCTCCTGGCTGCTTTGATGTC	NM_001024589
		R: CATTGGCGAGCTGCTGTGTG	Cano *et al.*, 2013
IL-1β	Interleukin-1β	F: ATGCTGAAGGCTCTCCACCTC	NM_214055
		R: TTGTTGCTATCATCTCCACCTC	Pierron *et al.*, 2016b
IL-8	Interleukin-8	F: GCTCTCTGTGAGGCTGCAGTTC	NM_213867
		R: AAGGTGTGGAATGCGTATTTATGC	Alasanne_Kpembi *et al.*, 2017

[1] F = forward; R = reverse.

Statistical analysis

For *in vivo* experiments, all data from animals (n=12) were subjected to ANOVA specific for repeated measures using the Mixed procedure (PROC GLM in SAS Institute, Cary, NC, USA). Sources of variation included litter, time, and their interactions. Specific treatment comparisons were made using Fisher's protected least significant difference test with comparisons of $P<0.05$ considered significant.

For explant experiments, all statistical analyses from explants were performed using Graph Pad Prism 4.0. Differences between the experimental groups were evaluated using one-way analysis of variance (ANOVA) followed by Bonferroni post-test (which allows comparison of all pair of groups). All data were expressed as mean ± standard error of the mean (SEM). A *P*-value below 0.05 was considered as significant.

3. Results

Effect of *Saccharomyces cerevisiae* RC016 on growth performance

The animals remained healthy throughout the experiment and diarrhoea was not detected in any of the pigs. When analysing the growth performance, significant differences between control animals and those receiving *S. cerevisiae* RC016 were observed for all parameters. An increase in daily weight gain in piglets that received the yeast strain after 15 d of feeding was observed (Figure 1). No differences at 15 d of feeding in total weight gain between the two studied groups were observed. However, at 18 and 22 d after feeding, an increase in this variable, influenced by the administration of yeast, was observed (Table 3). The FCR decreased in animals that received *S. cerevisiae* RC016, indicating a more efficient use of feed. In addition, improvement in the carcass weight and carcass performance was observed in animals that received only the yeast strain (Table 4).

Effect of *Saccharomyces cerevisiae* RC016 on intestinal histomorphometry

The results obtained from the morphometric studies are presented in Table 5. The oral administration of *S. cerevisiae* RC016 to weaned piglets did not induce change in the villus width and length and in the crypt depth. The number of goblet cells was increased in animals that received yeast administration compared to animals that did not receive the yeast strain (control group) (Table 5).

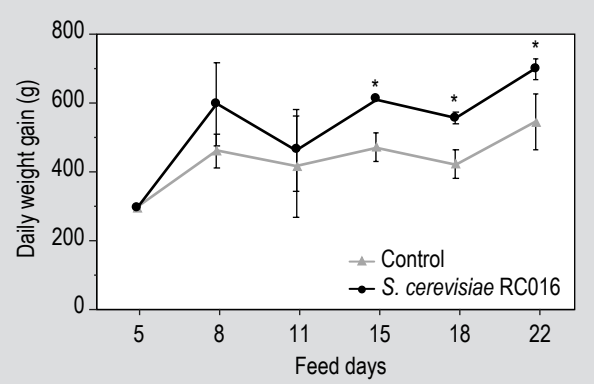

Figure 1. Daily weight gain of twelve pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *Saccharomyces cerevisiae* RC016 (1 g/kg). The data represent media (n=6) in g. (*) Indicate significant differences ($P<0.05$) between control and *S. cerevisiae* RC016 group.

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Table 3. Total weight gain at 15, 18 and 22 feed days with *Saccharomyces cerevisiae* RC016 and basal diet.[1]

Feed days	Control	*S. cerevisiae* RC016	Statistics	
			Residual error	*P*-value
15	5.67[a]	6.29[a]	0.46	0.01
18	7.01[a]	7.96[b]	0.46	0.01
22	8.15[a]	10.06[b]	0.46	0.01

[1] A total of twelve pigs were randomly assigned to individual pens. During the feeding period pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *S. cerevisiae* RC016 (1 g/kg). The data represent mean (n=6) in kg. Values within a row with different superscripts differ significantly.

Table 4. Feed conversion ratio, carcass (kg) and performance carcass (%) of piglets fed with standard diet and diet added with *Saccharomyces cerevisiae* RC016 for 22 days.[1]

Growth parameters	Control	*S. cerevisiae* RC016	Statistics	
			Residual error	*P*-value
Feed conversion rate	1.320[a]	1.230[b]	0.002	0.050
Carcass	10.500[a]	12.300[b]	2.480	0.050
Performance carcass	73.100[a]	85.600[b]	18.080	0.050

[1] A total of twelve pigs were randomly assigned to individual pens. During the feeding period pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *S. cerevisiae* RC016 (1 g/kg). The data represent mean (n=6). Values within a row with different superscripts differ significantly.

Table 5. Morphometric measurements in samples of small intestine of piglets fed with standard diet and diet added with *Saccharomyces cerevisiae* RC016 for 22 days.[1]

Morphometric measurement	Control	*S. cerevisiae* RC016	Statistics	
			Residual error	*P*-value
Crypt depth, µm	235.68[a]	214.83[a]	41.79	0.05
Villus length, µm	211.20[a]	203.97[a]	41.14	0.05
Villus width, µm	118.65[a]	120.80[a]	15.01	0.05
Goblet cells (no. cell/villus)	6.89[a]	9.94[b]	12.65	0.05

[1] A total of twelve pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *S. cerevisiae* RC016 (1 g/kg). The data represent mean (n=6). Values within a row with different superscripts differ significantly. Morphometric measurements taken from the intestinal histological sections of the small intestine (duodenum) from animals.

Effect of *Saccharomyces cerevisiae* RC016 on determination of s-IgA in intestinal fluid

The administration of *S. cerevisiae* RC016 demonstrated the s-IgA increase in intestinal fluid ($P<0.05$) in the small intestine of pigs after 22 d administration compared to the control group (Figure 2).

Effect of *Saccharomyces cerevisiae* RC016 on the number of intestinal lactobacilli and enterobacteria

The administration of *S. cerevisiae* RC016 during 22 d to healthy pigs resulted in an increase of lactobacilli counts mean log cfu (6.16±0.19) compared to the control group

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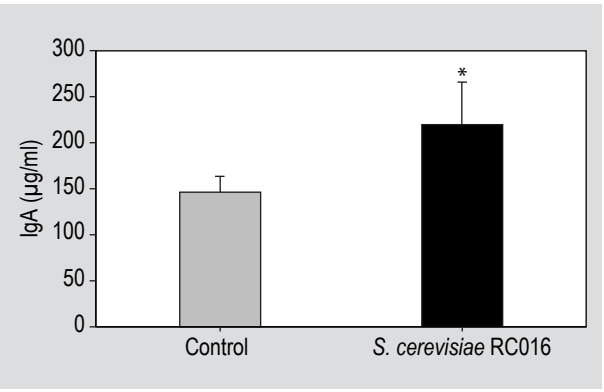

Figure 2. Secretory IgA (s-IgA) concentrations in samples of small intestine of twelve pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *Saccharomyces cerevisiae* RC016 (1 g/kg) for 22 days. The data represent media ± standard deviation (n=6) in µg/ml of intestinal contents. (*) Indicates significant differences with *P*<0.05.

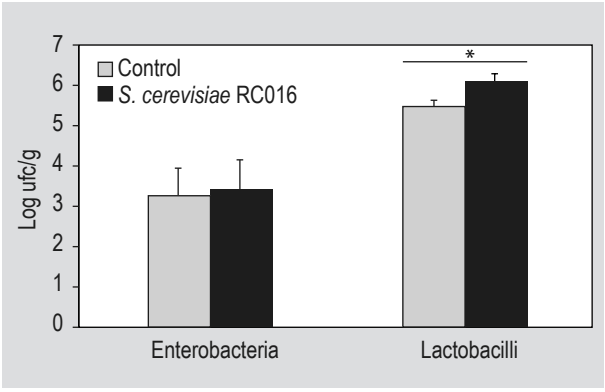

Figure 3. Enterobacteria and lactobacilli counts in samples of large intestine (caecum) of twelve pigs had *ad libitum* access to water and feed supplemented with (n=6) and without (n=6) the inclusion of *Saccharomyces cerevisiae* RC016 (1 g/kg) for 22 days. Colony counts are expressed as log_{10} numbers of bacteria per gram of caecum. Each bar represents the mean ± standard deviation (n=6). (*) Indicates significant differences with *P*<0.05 between control and *S. cerevisiae* RC016 groups.

(5.50±0.17) (Figure 3). No significant changes were observed for the enterobacteria counts, comparing the two groups.

Effect of *Saccharomyces cerevisiae* RC016 on cytokines gene expression in jejunal explants

To study the effect of *S. cerevisiae* RC016 on the intestinal inflammation, explants were treated with DON, known to induce an intestinal inflammation (Payros *et al.* 2016), *S. cerevisiae* RC016 or both. The expression of several pro-inflammatory genes was assessed by qPCR (Figure 4). As expected, DON increases significantly the expression of IL-1β, IL-22, and IL-8 pro-inflammatory cytokine genes as compared to control group. An increase of the chemokine gene expression CCL20 was also observed. By contrast, treatment of jejunal explants with *S. cerevisiae* RC016 alone did not induce an increase in the expression of these genes. The presence of the yeast strain, significantly decrease the DON-induced inflammation as measured by the expression of IL-1β, IL-8 and CCL-20.

4. Discussion

The weaning period in young pigs is frequently associated with physiological, environmental and social challenges that lead to subsequent diseases and other production losses. Technological improvements in housing, nutrition, health, and management have been used to minimise some of the adverse effects of weaning stress. Recent concerns regarding antibiotic resistance have resulted in a need for alternative strategies to improve animal production and health without antibiotics. Among the proposed alternatives, probiotics are good candidates that improve digestive mechanisms, stimulate the immune system and improve weight gain (Buts, 2009; Chang *et al.*, 2001; Dogi *et al.*, 2008).

Commercial species of probiotics are usually isolated from the intestinal microbiota of the intended consumer (for example human, chicken or pig) and selected on the basis of criteria such as resistance to stomach acids and bile salts, ability to colonise in the intestine or antagonism of potentially pathogenic microorganisms (Verdenelli *et al.*, 2009). *S. cerevisiae* RC016 was isolated from gut of healthy pigs and demonstrated beneficial properties, such as co-aggregation and inhibition of enteric pathogens, as well resistance to gastrointestinal conditions (Armando *et al.*, 2011).

Histological evaluation of duodenum and ileum indicated no effect of dietary supplementation with *S. cerevisiae* RC016 on villus length, width and crypt depth. These results are in agreement with previous studies, which reported that yeast fails to induce morphological alteration of the intestinal mucosa (Buts *et al.,* 1986; Van der Peet-Schwering *et al.,* 2007). However, it was also reported that administration of *S. cerevisiae* had a positive effect on nursery pigs by improving jejunal villus height and villus height:crypt depth ratio (Gao *et al.*, 2008; Shen *et al.*, 2009). Goblet cells reside throughout the length of the small and large intestine and are responsible for the production and maintenance of the protective mucus layer by synthesising and secreting high-molecular-weight glycoproteins known as mucins. The mucus barrier is the first line of host defences against noxious agents and infections. In the present work, feed supplementation with *S. cerevisiae* RC016 reinforces this barrier by increasing the number of goblet cells. These results are in accordance with other studies reporting that some probiotics enhance mucin secretion and MUC2 gene expression in rat colon or in HT-29 cell line and increase the number of goblet cells in mice duodenum (Caballero-Franco *et al.*, 2007; De Moreno de LeBlanc *et al.*, 2008; Duary *et al.*, 2014).

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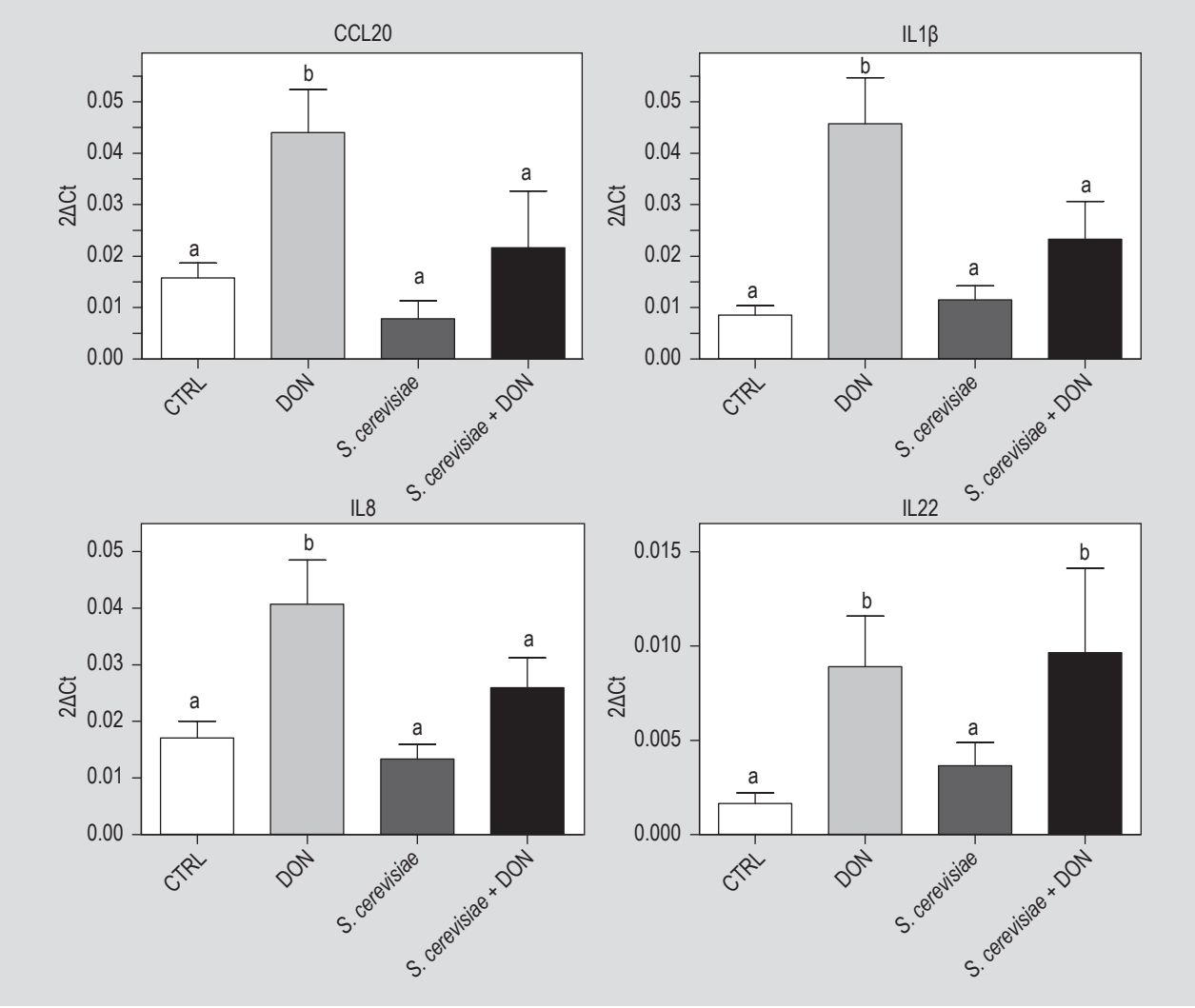

Figure 4. Expression of mRNA encoding for cytokines in small intestine of jejunal explants. Jejunal explants were exposed with: complete William's Medium E (CTRL); with 10^7 cfu/ml *Saccharomyces cerevisiae* RC016 in complete medium; pre-treated with complete medium 1 h and then exposed to 10 µM deoxynivalenol (DON) in complete medium; or pre-incubated 1 h with 10^7 cfu/ml *S. cerevisiae* and then exposed to 10 µM DON in complete medium. After 3 h of incubation, gene expression was assessed. Relative expression of mRNA encoding for pro-inflammatory cytokines and chemokines was measured by RT-qPCR. Data are normalised to housekeeping gene RPL32 and expressed, in arbitrary unit, as mean and standard error of the mean of explants from 6 animals. Columns having different superscripts differ significantly (one-way ANOVA with Bonferroni's multiple comparisons).

S. cerevisiae RC016 administration increased the levels of s-IgA in the intestinal lumen. the main mechanism of protection given by the gut associated lymphoid tissue is humoral immune response mediated by s-IgA, which contributes to host defence against intestinal pathogens, warrants that pro inflammatory processes are kept under control, preserving the integrity and functionality of the epithelial barrier (Brandtzaeg, 2013; Cerrutti *et al.*, 2011, Corthesy, 2013). Therefore, the stimulation of immune cells to secrete s-IgA is often considered a desirable property in the screening of probiotic microorganisms. Several reports have demonstrated that different yeast strains are able to increase S-IgA (Ashraf and Shah, 2014; Jiang *et al.*, 2015; Thomas and Versalovic, 2010).

Post-weaning disorders in pigs result in gastrointestinal alterations, such as major changes in the adapting enteric microbiota; while populations of *Lactobacillus* remain stable and abundant before weaning, their numbers drop significantly after weaning in conjunction with a marked increase in enterobacteria counts (Konstantinov*et al.*, 2004). Lactobacilli and enterobacteria have been traditionally selected as microbial groups with a particular significance for gut health. The presence of lactobacilli in the gastrointestinal tract of pigs is believed to be beneficial for the animal, whereas the presence of enterotoxigenic bacteria and *Salmonella* is associated with diarrhoea and other digestive disorders (Jonsson and Conway, 1992). In the present work, post-weaning administration of *S.*

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cerevisiae RC016 increased the number of lactobacilli while the enterobacteria population remained stable. Hence, the ability to modulate these two microbial groups can be an important target for dietary interventions targeting porcine gastrointestinal tract disturbances.

Improving the performance and health of the animals is the major aim of using probiotics in pigs. The results obtained in the present work should be interpreted with caution due to the small number of animals used per group. However, despite the small number of animals per group a positive trend was observed with regard to animal performance. In order to confirm this trend, future studies will be carried out with a higher number of animals. The parameters of PWG and TWG weight, carcass weight and FCR could be improved by the administration of live *S. cerevisiae* RC016 during the first three weeks after weaning. The conversion of high quality feed into weight gain is of absolute importance in modern pig production, especially as feed costs increase. Feed conversion is the ability of livestock to turn feed mass into body mass. The smaller the FCR index the better-feed conversion. Similar effects were obtained by other authors with a single probiotic strain or with probiotic complexes diet. In these cases, the doses of the probiotic strain used were 1×10^{10} cfu/g feed (Che *et al.*, 2017; Kiros *et al.*, 2018; Trckova *et al.*, 2014) or 3.3×10^{9} cfu/g feed (Hancox *et al.*, 2015). Is important to remark that the beneficial effects demonstrated in the present work were seen when the probiotic was administered at a smaller dose (1×10^{7} cfu/g feed) than the used in the above-mentioned studies. A higher dose involves more cost and is not profitable for the producer.

Some studies reported that the administration of probiotics did not have an impact on the growth performance of the animal (Broom *et al.*, 2006; Gagnon *et al.*, 2007; Simon *et al.*, 2003; Taras *et al.*, 2006; Veizaj-Delia *et al.*, 2003). These inconsistent reports may be due to several aspects such as strains of probiotic microorganisms, dose level, diet composition, feeding strategy and interaction with other dietary feed additives.

In the context of reducing the number of experimental animals, intestinal explants represent a powerful model and have been introduced as a model to test intestinal integrity since allows to preserving normal histological structure *in vitro* (Nietfeld *et al.*, 1991). As previously described (Cano *et al.*, 2013; García *et al.*, 2018; Pierron *et al.*, 2016a), DON increases significantly the expression of pro-inflammatory cytokine genes in jejunal explants and a decrease in these gene expression was observed with the pre-incubation of *S. cerevisiae* RC016. Previous studies demonstrated the ability of this yeast strain to modulate the gut immune system in healthy BALB/c mice (García *et al.*, 2016). In this work, we use *ex vivo* model for study the ability of *S. cerevisiae* RC016 to counteract the inflammation of the intestine during the first weeks after weaning. According to our results, the yeast strain has anti-inflammatory potential to reverse/prevent the harmful consequences of weaning.

This work was carried out to provide knowledge for the future development of a new feed additive, with both probiotic and mycotoxin adsorbent properties, intended to improve gut health and growth performance while minimising the use of antibiotic as growth promotor.

5. Conclusions

In recent years, it has been elucidated that a healthy gut is the most important precondition for transforming nutrients into performance. In this study, *S. cerevisiae* RC016 demonstrated to improve intestinal health, which can then lead to better general health. Also, the addition of the yeast did not produce negative effects on growth performance of the pigs. In conclusion, *S. cerevisiae* RC016 is a probiotic strain with the ability to modulate the gut ecosystem in weaned piglets, minimising the use of antibiotic as growth promotor. The strain's incorporation to the animal's diets as a novel feeding strategy would favour for the long-term sustainability of the pig industry.

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Conflict of interest statement

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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