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Saccharomyces boulardii RC009 as an alternative to prophylactic antibiotics: effects on broiler chickens, performance, and gut microbiome

A.P. Magnoli[1,2*], M.J. Luna[1,2], L.J. Giordano[2,3], M.C. Isgro[2,4], V.L. Poloni[2,5], D.G. Ceschin[2,6], J. Parada[2,4] and L.R. Cavaglieri[2,5]

[1]Department of Animal Production, National University of Río Cuarto, Córdoba, [2]National Scientific and Technical Research Council (CONICET) Buenos Aires, [3]Center for Translational Medicine Research "Severo R. Amuchástegui" (CI-METSA), National Scientific and Technical Research Council (CONICET), Córdoba, [4]Department of Animal Pathology, National University of Río Cuarto, Río Cuarto, Córdoba, [5]Department of Microbiology and Immunology, National University of Río Cuarto, Córdoba, [6]University Institute of Biomedical Sciences of Córdoba (IUCBC), Córdoba, Argentina

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Correspondence:
A.P. Magnoli
amagnoli@ayv.unrc.edu.ar

Abstract

This study evaluates the effects of supplementing broiler chicken feed with the probiotic *Saccharomyces cerevisiae var. boulardii* RC009 as a sustainable alternative to prophylactic antibiotics. The focus was on productive performance, gut microbiome composition, and the time to reach slaughter weight. The trial involved 77,200 one-day-old Cobb-500 broiler chickens housed on 5 floor pens. Two groups were compared: Control (Basal Diet + antibiotics) and Probiotic (Basal Diet + *S. boulardii* RC009, without antibiotics). The probiotic was administered from day 0 until the birds reached 3 kg. The Probiotic group showed notable improvements: males reached 10.97% higher slaughter weight and achieved market size two days earlier than controls. Histomorphological analysis indicated preserved intestinal health, with no significant differences between groups. 16S rDNA sequencing revealed that the probiotic significantly altered the gut microbiota. Although overall microbial diversity slightly decreased, there was a marked enrichment of methanogenic Archaea, particularly *Methanobrevibacter* and other *Methanobacteriales*, alongside a reduction in dominant bacterial families such as *Ruminococcaceae* and *Lachnospiraceae*. These findings suggest that supplementation with *S. boulardii* RC009 improved broiler performance by increasing slaughter weight and reducing time to market. The rise in methanogenic Archaea suggests enhanced redox balance and potential increases in Short-Chain Fatty Acid (SCFA) production, contributing to better digestibility and anti-inflammatory effects. These results support *S. boulardii* RC009 as a viable, sustainable alternative to antibiotic growth promoters in poultry farming.

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Introduction

The gastrointestinal tract (GIT) of chicks can be colonized by microbiota as early as the embryonic stage, during egg formation in the oviduct, and while the chick is moving through the reproductive tract (1). Following hatching, the newly developing bacterial communities face various challenges, as a wide range of environmental factors and genetic influences can impact the composition of the GIT microbiome (2,3). All these factors, to varying degrees, affect the state of the GIT microbiota and, consequently, the processes of digestion, growth, viability, and feed conversion in birds.

For decades, farmers have used a significant number of growth-promoting antibiotics (GPA) at sub-therapeutic doses to mitigate the negative effects of infections by

suppressing and inhibiting pathogenic bacteria in the digestive tract, thereby creating a more favorable environment for beneficial intestinal bacteria. The misuse of GPA in animal production has led to concerns about antibiotic resistance and its impact on public health. An alternative to their replacement is the use of natural feed additives such as organic acids, probiotics, prebiotics, and enzymes, which reflects a broader shift toward more responsible and sustainable animal husbandry practices (4,5). Studies have shown that many of these alternatives, such as probiotics and prebiotics, can enhance gut health, improve nutrient absorption, and consequently boost growth performance in animals (6,7).

The Food and Agriculture Organization and the World Health Organization have defined probiotics as live microorganisms that, when provided in adequate amounts, can improve the intestinal microbial balance and confer health benefits to the host (8). Different authors have shown that probiotic supplementation in broiler chickens' diets can improve growth, conversion efficiency, nutrient utilization, increase microbial balance, immune system regulation, and carcass weight (9-11). The addition of *Saccharomyces boulardii* RC009 as a probiotic has been previously demonstrated to provide beneficial effects, improving the health and productivity of broiler chickens (12,13). Subsequently, Parada *et al.* (14) demonstrated that the use of *S. boulardii* RC009 as a nutritional feed additive was able to substitute the prophylactic use of antibiotics and improve the productive performance and health of post-weaning piglets. Also, they demonstrated that pigs consuming the *S. boulardii* RC009 improved both diversity and microbial richness in the gut microbiota (15). *Saccharomyces cerevisiae* is a yeast that can inhibit the growth of pathogens in the GIT. It produces compounds such as lactate, lactic acid, and volatile fatty acids, which help lower the pH in that area. This creates a less favorable environment for pathogens, which can be beneficial for gut health (5).

Therefore, this study aimed to evaluate the effects of supplementing broiler chicken feed with *Saccharomyces cerevisiae* var. *boulardii* RC009 as an alternative to prophylactic antibiotics. The focus was on productive performance, gut microbiome composition, and the time it takes to reach slaughter weight.

Based on previous studies (12-15), we hypothesize that supplementation with *S. boulardii* RC009 will result in productive performance equivalent to or better than the growth-promoting antibiotic group, will significantly improve the feed conversion ratio, and reduce the time to reach slaughter weight compared to the control, and increase the diversity and richness of the gut microbiome while suppressing the relative abundance of pathogenic bacteria.

This study presents an integrated and comprehensive evaluation of the inclusion of *Saccharomyces boulardii* RC009 in broiler chickens, simultaneously addressing performance, microbiome, and economic variables (time to slaughter weight) in the context of antibiotic substitution.

Materials and methods

Ethical approval

The work protocol and the techniques used were approved by the Animal Ethics Subcommittee of the Committee on Ethics in Scientific Research of the National University of Río Cuarto (383/30/06/22).

Probiotic additive

Probiotic yeast (YEA) strain was stored in the culture collection of the Department of Microbiology and Immunology at the National University of Río Cuarto, Córdoba, Argentina (*Saccharomyces boulardii* RC009 GenBank N°MH266045). It constitutes a technological license that was transferred from the public-academic sector (UNRC-CONICET) to the private sector (BIOFEED TECH SAS-BIOFACTORY SAS) within the framework of the International Nagoya Protocol, which was approved for commercial use. The strain was maintained at -20°C in 50% (v/v) skim milk. A transfer was made from the frozen stock to Yeast-Peptone-Dextrose broth (YPD-20 g/L glucose, 10 g/L peptone, 10 g/L yeast extract) and incubated for 24 h at 37°C to obtain a working culture.

Probiotic biomass production at pilot scale

YEA biomass production was performed in a bioreactor (BioFlo 2000 fermentor - New Brunswick Scientific Co., Inc., Enfield, CT, USA) operated at 4 x g at 28ºC, 300 rpm for 12 h 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 was centrifuged at 1000 x g at 4°C for 10 min. The concentrated biomass was used for subsequent studies.

The concentration of *S. boulardii* RC009 was 1×10^9 CFU/g (12-15). The probiotic additive was mixed with the corresponding diet to reach 1×10^{11} CFU/t feed.

GPA: Avilamycin 10%, commercially obtained, was used in 100 g/t feed.

Broiler chickens and housing

The assay was conducted at a poultry farm owned by INDACOR S.A., located in Juárez Celman station city, Colón department, Córdoba province, Argentina. A total of 77 200 broiler chickens, day-old Cobb-500 commercial line, were distributed among five (5) floor pens according to sex. Before placement, all chicks were visually examined for health, and inferior chicks were excluded from the trial. The concrete floor was covered with ~3 in (7.6 cm) clean softwood wood chips, and ventilation was provided by negative pressure with fans. Heat was provided by gas-fired brooders; water and feed were offered *ad libitum* through nipple drinkers and tube feeders, respectively. Birds were

managed according to the Cobb recommendations (16). The composition of the basal diet starter (days 0–10), grower (days 10–20), and finisher (days 20–30) diets included corn as the main cereal, and soya and soybean cake as protein concentrates to meet the nutrient requirements for broiler Cobb-500 (BD) (Table 1).

Table 1. Composition (g/kg diet) of the Basal Diet (BD)

Ingredient	Diet		
	Starter	Grower	Finisher
Macro ingredient			
Yellow corn	443.0	495.0	530.0
Soybean Expeller	410.0	398.0	380.0
Soybean heat-treated	80.0	50.0	50.0
Meat and bone meal	62.0	32.0	18.0
Micro ingredient			
Vitamin and mineral mix[1]	3.0	2.7	2.0
NaCl	4.0	4.0	3.58
calcium carbonate	6.66	6.66	6.0
Liquid Methionine	4.46	4.93	4.6
L-Lysine	1.96	1.8	1.53
L Threonine	1.0	1.6	0.8
Nutritional levels (g/Kg)			
Crude protein	249.3	233.1	221.5
Crude fat	83.2	76.8	74.5
Crude fiber	43.2	41.7	41.2
Calcium	11.0	10.0	8.0
Total phosphorus	8.12	7.59	6.71
Lysine	15.09	14.51	13.57
Methionine	7.31	7.48	7.12
Tryptophan	3.04	2.83	2.72
Metabolizable energy (Mcal/kg)	3175	3189	3220

[1] Premix contained the following per kg powder: calcium 10.2%, starch 0.016%, crude fibre 0.012%, vitamin A 1 600 000 IU, vitamin D3 320 000 IU, vitamin E 4 800 IU, vitamin B1 320 mg, vitamin B2 800 mg, vitamin B6 640 mg, vitamin B12 3 200 µg, vitamin K3 320 mg, pantothenic acid 1 600 mg, niacin 6 400 mg, biotin 24 000 µg, folic acid 160 mg, choline chloride 24 000 mg, iron 6 400 mg, iodine 160 mg, copper 1 600 mg, manganese 12 800 mg, zinc 9 600 mg, selenium 24 mg.

Study Design

The five (5) pens were assigned according to sex. The groups were: Control: (Basal diet (BD) with GPA, pen 1: 19,500 females and pen 2: 19,500 males; Probiotic: BD without GPA with *S. boulardii* RC009; pen 3: 19,800 females, pen 4: 10,000 males, and pen 5: 8,400 males. Application of the tested product began on day 0 and continued until the animals reached 3 kg live weight. All birds were vaccinated against coccidiosis.

Data Collection

Productive parameters

Chicks were weighed at the start of the trial (day 0) and every week thereafter. The daily weight gain (DWG-g) was calculated by the difference between the final and initial weight by dividing the weight by the number of days of assay, daily feed consumption (DFC-g) estimated as follows: the amount of feed left in the feeder was weighed and the difference was divided by the number of days of assay and the conversion rate (CR) was calculated as the relationship between the DFC and DWG. Morbidity and mortality were recorded daily, and the slaughter weight of each group was recorded.

Anatomopathological examinations

When the chickens reached 3 kg, 20 broiler chickens per pen were randomly selected and euthanized. Changes in the internal organs were assessed according to the following scale: no visible changes; visible minor changes in the form of hyperaemia; medium-grade hyperaemia of the mucous membrane with minor petechiae observed; and strongly expressed hyperaemia of the mucous membrane, marked petechiae, and enlargement and swelling of internal organs.

Histomorphometric parameters

Tissue samples (duodenum) for histology were taken and processed using the methodology described by Poloni *et al.* (17). The morphometric measurements taken from the intestinal histological sections (length, width of villus, and intestinal crypt depth) were estimated according to Luna *et al.* (18). The absorptive surface area of the duodenal villus was estimated by considering a villus as a cylindrical structure (18). Villus absorptive surface area was calculated using the following formula according to Sohail *et al.* (19): Villus absorptive surface area = $2\pi \times$ (average villus width/2) × villus height. Cecal contents and cloacal (fecal) samples were aseptically collected from 10 broiler chickens by pen and transferred to sterile Whirl-Pak plastic bags (Nasco, Fort Atkinson, WI) and test tubes, respectively. They were immediately frozen (-20°C) and transported to the laboratory for microbiota analysis.

Sample collection and genomic DNA extraction

At the end of the study, fecal samples were taken by anal stimulation. Samples were refrigerated, transported immediately, and frozen at -80°C until analysis. These samples were processed in pools (n=20) for each group, and DNA was extracted using the QIAamp DNA Stool Mini Kit (Qiagen) for metagenomics studies. Briefly, the extracted and purified DNA was sent to the Macrogen sequencing service (Korea) to perform deep sequencing (Next Generation Sequencing -NGS), from amplifications of the V3 and V4 regions of the 16S rDNA, using the standardized primers by Macrogen: Bakt_341F:

CCTACGGGNGGCWGCAG, and Bakt_805R: GACTACHVGGGTATCTAATCC.

Sequencing data analysis

Paired-end reads were merged using FLASH (V1.2.7) (20). To obtain clean and effective tags, quality filtering on raw sequences was performed according to the QIIME quality control process (21), and the reference database (Gold database) was used to detect and remove chimera by UCHIME algorithm (UCHIME Algorithm) (22). Taxonomic classification was performed using Kraken2 v2.1.3 (https://doi.org/10.1186/s13059-019-1891-0) with the SILVA v138 database. Alpha diversity (Simpson index) was calculated using the vegan package in R v4.4.3. Taxonomic composition bar plots and Venn diagrams were generated using ggplot2 v3.5.2 (23) in R.

Statistical analysis

The productive parameters were expressed as the mean ± standard deviation (SD) and analyzed using a complete factorial ANOVA. The means were compared according to Fisher's least significant difference test (LSD) ($P<0.05$) (24). The analysis was carried out using the InfoStat program (25).

Results

Productive parameters

The productive parameters evaluated did not show significant differences between the groups ($p \geq 0.05$) (Table 2). The weight gains at 42 d-age for females and males treated with the *S. boulardii* RC009 was 4.33% and 5.40% respectively, compared with females and males in the control group. Notably, while the males of both groups reached slaughter weight at the same time, males given the *S. boulardii* RC009 had a 10.97% higher slaughter weight compared to the control group. In contrast, broiler chickens receiving the probiotic (and no GPA) reach slaughter weight two days before the broiler chickens in the control group (who received GPA). Regarding the conversion rate, it was similar in both groups. When compared to animals receiving GPA but no probiotics, broiler chickens supplemented with *S. boulardii* RC009 exhibited a positive trend across the evaluated productive parameters.

Anatomopathological examinations

There were no macroscopic changes in the livers or other internal organs. In both groups, erosions or mucosal gizzard lesions were noted (Figure 1A). In control chickens, various degrees of changes related to congestion of the mucous membrane of the small intestine were observed; these changes observed in the control group may have indicated the influence of coccidian (Figure 1B).

Small intestine histomorphometry

The histomorphometric variables did not show significant differences between the groups with *S. boulardii* RC009 and GPA ($P \geq 0.05$) (Table 3) (Figure 2A and B). However, broiler chickens fed the *S. boulardii* RC009 showed a greater surface area of absorption and lower villus height.

Table 2: Productive parameters in broiler chickens obtained with *S. boulardii* RC009 and antibiotic on weekly weight gain (WWG), daily weight gain (DWG) per pen and sex (kg), and conversion rate (CR)

Groups	Control		*S. boulardii* (RC009)		
Pens	1	2	3	4	5
N	19500	19500	19800	10000	8400
Sex	F	M	F	M	
Days	Weekly gain weight (M ± SD)				
4	0.104 ± 0.020	0.101 ± 0.008	0.097 ± 0.010	0.122 ± 0.008	
7	0.153 ± 0.06	0.151 ± 0.015	0.153 ± 0.006	0.158 ± 0.008	
14	0.423 ± 0.013	0.415 ± 0.010	0.383 ± 0.015	0.495 ± 0.005	
21	0.849 ± 0.018	0.874 ± 0.059	0.859 ± 0.032	0.926 ± 0.011	
28	1.401 ± 0.050	1.505 ± 0.034	1.396 ± 0.042	1.569 ± 0.027	
42	2.746	2.925	2.865	3.083	
Slaughter	47	44	45	44 - 45	
Slaughter weight	2.997	2.925	2.865	3.246	
Weight between F and M to slaughter	2.96		3.12		
DWG	65.15	66.48	63.82	72.95	
DWG between F and M	65.82		69.66		
CR	1.863		1.860		

N: number of animals; DWG: (daily weight gain); CR: conversion rate; M: (males); F: (females); M ± SD (means± standard deviation).

Table 3: Influence of probiotic *S. boulardii* RC009 on the histomorphometric parameters in broiler chickens

Groups	Duodenum Villus (µm)				
	Height	Width	Crypt Depth	Absorptive Surface Area	Ratio
Control	1075.11±220.67[a]	181.52±71.37[a]	171.63±45.63[a]	595698.5±200335[a]	6.7±2.2[a]
S. boulardii RC009	969±173.9[b]	200.9±67.9[a]	165.3±55.6[a]	621775.9±263294.2[a]	6.3±1.7[a]

[a,b] Values (Mean+SD) differ significantly (P≤0.05) bearing different alphabets in the same column.

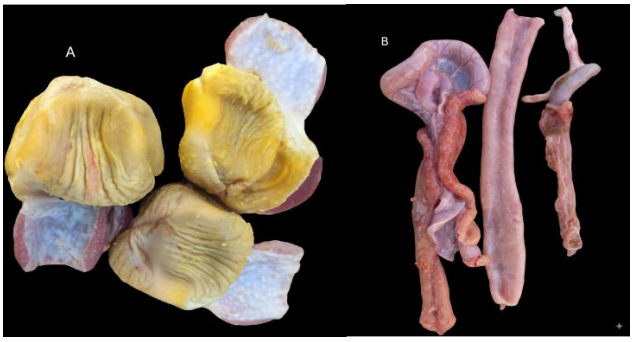

Figure 1: Anatomopathological examinations of the gizzard (A) and small intestine (B) of both groups.

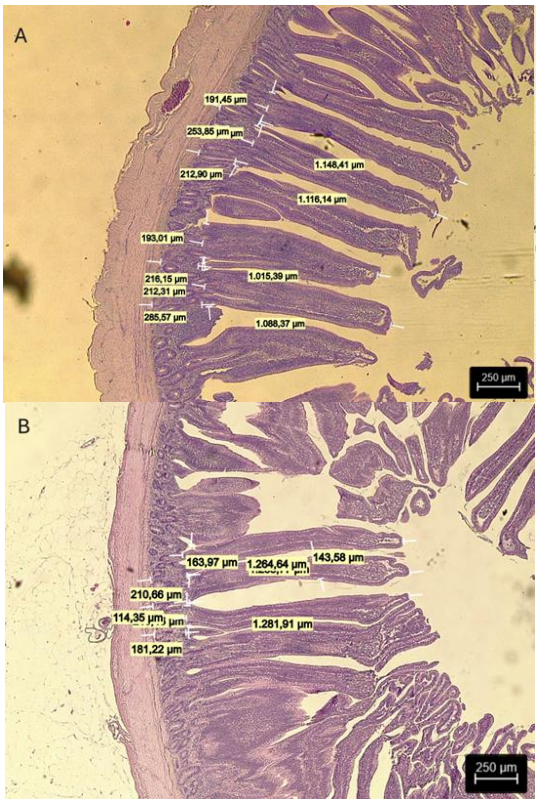

Figure 2: Photomicrograph of broiler chicken duodenal sections at 47 days of assay, stained with Hematoxylin-Eosin 20x. (A) Probiotic: basal diet (BD -without GPA plus *S. boulardii* RC009. (B): Control: basal diet (BD -control with GPA (Avilamycin 100g/T)).

Microbial diversity of the intestine

The 16S rDNA gene sequencing allowed for monitoring the diversity of the microbial community in the intestine. Alpha-diversity analysis revealed that probiotic supplementation (L2 - 3.255) was similar to the microbial diversity of the control one (L1 - 3.355). A Venn diagram (Figure 3) illustrates a large core set of microbial taxa common to both groups, but also unique taxa associated with each. The control sample (L1) had a slightly higher total number of unique taxonomic units compared to the treated sample (L2). Specifically, L1 harbored 49 unique taxa that were not present in L2, while L2 had 35 unique taxa that were not present in L1. While 164 taxa were shared between the groups, the net loss of 14 unique taxa from L1 to L2 contributes to the observed reduction in diversity in the treated group. At the phylum level, *Bacteroidetes* and *Firmicutes* were the most abundant (Figure 4). No statistically significant differences were observed between groups, though there was a trend towards decreased abundance of *Proteobacteria* and *Verrucomicrobiota* in the probiotic group. The probiotic group profoundly modulated the microbial community composition at the family level (Figure 4). *Ruminococcaceae* and *Lachnospiraceae* were most abundant in the control sample. However, the probiotic group significantly promoted the growth of *Methanobacteriaceae*, which was almost negligible in the control. Due to this surge, the relative abundances of *Ruminococcaceae* and *Lachnospiraceae*, dominant in control, were proportionally modulated in the probiotic sample. Changes in these families, both important in carbohydrate fermentation, could suggest altered dietary conditions or metabolic functions in the probiotic group. The most significant finding at the genus level was the substantial increase in *Methanobrevibacter* in L2 (Figure 5). Concurrently, there was a noticeable relative decrease in several bacterial genera more prominent in the control, including *Ruminobacter*, *Streptococcus*, *Prevotella*, and *Phascolarctobacterium*, indicating a clear shift in bacterial community structure. Interestingly, *Fibrobacter* showed an increase in L2. This demonstrates a major restructuring of the microbial community, with dramatic enrichment of *Methanobrevibacter*, an archaeon, at the expense of several common bacterial genera. The most profound effect of the probiotic group was the dramatic enrichment of the order *Methanobacteriales* in L2, consistent across family and genus-level analyses (Figure 5). This was accompanied by a significant reduction in the abundance of several dominant

bacterial orders found in the control, including *Lachnospirales*, *Oscillospirales*, and *Bacteroidales*, indicating a major shift in the bacterial component of the microbiome.

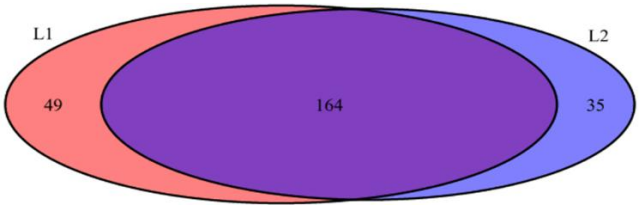

Figure 3: Venn diagram of microbial diversity between Control (L1) and Treated Groups (L2).

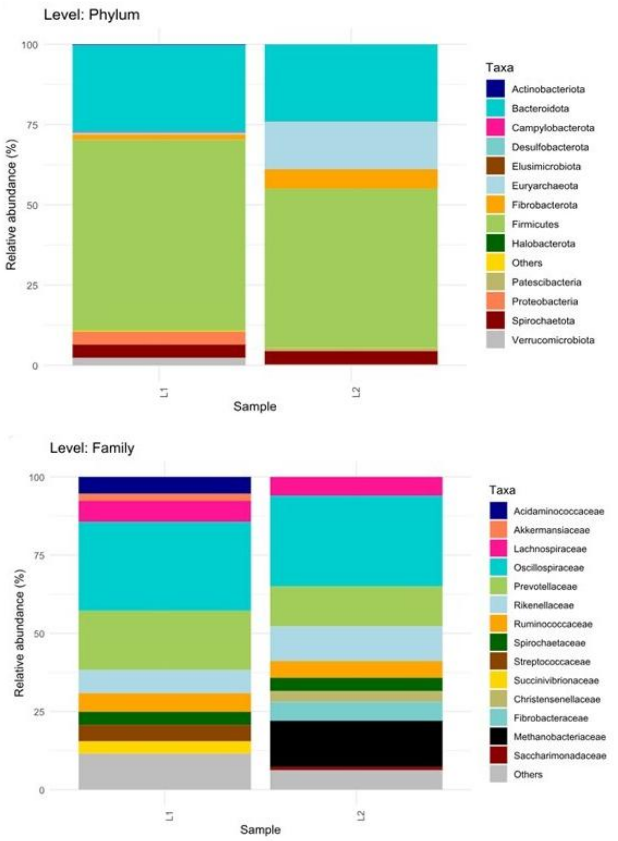

Figure 4: Relative abundance of microbial phyla and Taxonomic composition of the microbial community at the Family level in control group samples (L1) and treatment group samples (L2).

Some orders, like *Christensenellales* and *Fibrobacterales* appeared relatively more abundant in L2, suggesting potential resilience or even a favored status under the new conditions, even as overall bacterial diversity seemed compressed by the *Methanobacteriales* bloom. These results reinforce that the probiotic group created an environment highly conducive to the growth of *Methanogenic archaea*, causing a significant ecological shift and reshaping the entire microbial community structure. At the class level, the most prominent result was the very large increase in the relative abundance of *Methanobacteria* in L2 (Figure 5). This consistent, overarching theme across all taxonomic levels led to a relative reduction in the proportions of major bacterial classes highly abundant in the control, such as *Clostridia*, *Bacteroidia*, and *Bacilli.* While *Clostridia* remained significant in the probiotic sample, the community was heavily influenced by the massive presence of the archaeal class Methanobacteria, alongside notable increases in *Gammaproteobacteria* and *Fibrobacteria*.

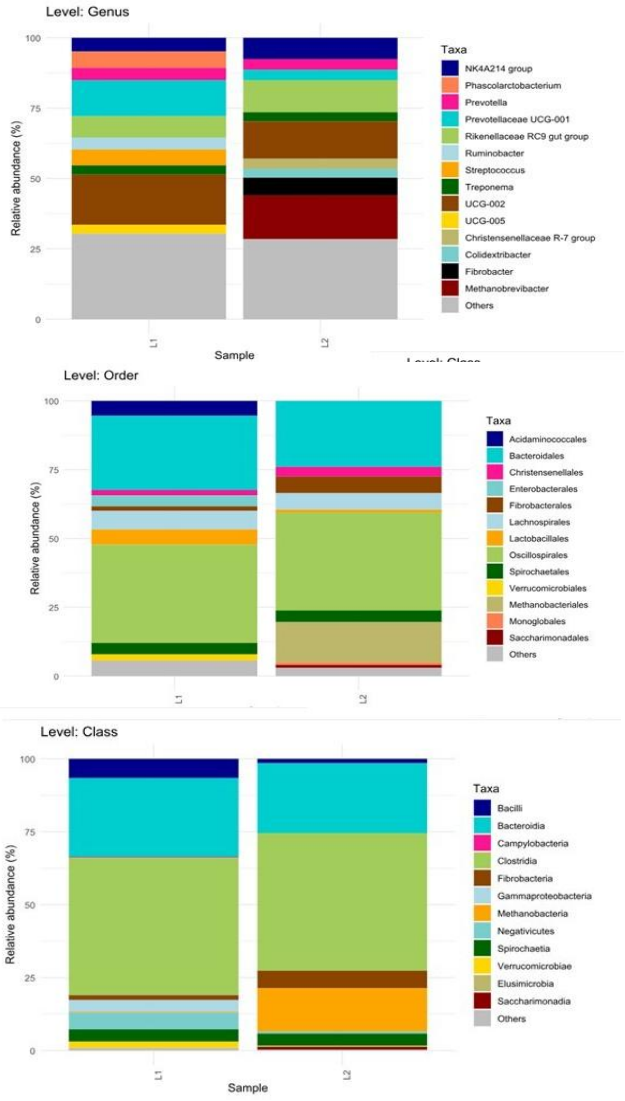

Figure 5: Relative abundance and composition of microbial Genera, Orders, and Classes in control group samples (L1) and treatment group samples (L2).

Discussion

The use of feed additives (in-feed antibiotics, probiotics, phytobiotics, etc.) has become widespread in the poultry industry (5). Antibiotics have been used for many years as growth promoters in animal production. The sub-therapeutic application of antimicrobials in animal production is the key driver of antimicrobial resistance. This is why the use of antibiotics in the farming sector for growth promotion has come under increasing scrutiny due to their effects. Therefore, there is a need to eliminate the use of antibiotics as growth promoters and to ensure the responsible use of antimicrobials in animal production systems (4).

This study aimed to assess the effects of supplementing *S. cerevisiae* var. *boulardii* RC009 as a nutritional feed additive, as an alternative to the GPA, on the productive performance of broiler chickens, the composition and modulation of their gut microbiome, and the time required to reach slaughter weight.

Probiotics enhance intestinal and overall health primarily through a multi-faceted approach. They re-establish microbial equilibrium by promoting beneficial bacteria while suppressing harmful ones (9). Key actions also include strengthening the gut barrier by binding to epithelial cells and improving the mucous layer by resisting pathogen invasion, modulating the host's immune system, and increasing the production of health-promoting metabolites (9).

According to this study, the addition of 1 x 10^9 CFU/g *S. boulardii* RC009 resulted in no significant differences in productive parameters compared to the control (avilamycin 10%) during the experimental period. This suggests that *S. boulardii* RC009 could replace GPA. On the other hand, although both male groups (control and probiotic) reached slaughter weight simultaneously, probiotic-fed males were heavier at slaughter than the control ones (26). Probiotics also accelerated female growth, with probiotic-treated females reaching slaughter weight two days earlier than the control group. This suggests that probiotics use could facilitate more breeding cycles per year, indicating a promising alternative for enhancing poultry production (9). These results partially agree with Roy and Ray (26), who observed an improvement in body weight gain and feed conversion ratio (FCR) when they fed chickens 20 g of *S. boulardii* (CNCM 1-1097) at a concentration of 2.0 × 10^{10} CFU/g per 100 kg feed. In the same way, Lin *et al.* (27) showed that dietary supplements with *Saccharomyces cerevisiae* hydrolysate (250-500 mg/kg) stimulate the growth of broilers. Different investigations have demonstrated the possible replacement of antibiotics as growth promoters by probiotics (10,14,15,26,28). The effects on production parameters could be due to *S. boulardii* contributing to digestion by secreting exogenous enzymes into the intestinal lumen, which increases the digestibility of dry matter and the metabolizable energy of the feed (9). Besides, previous studies have suggested a connection between increased microbiota diversity and improved productive performance and health status in broiler chickens (9,26,29).

The improved gut structure can enhance nutrient absorption and overall digestive function. Supplementation with *S. cerevisiae* has been shown to improve gut morphology in broilers, with increased villi height in the duodenum, jejunum, and ileum (9). This improved gut structure can enhance nutrient absorption and overall digestive function. In our studies, the histomorphometric data did not show differences between the groups with *S. boulardii* RC009 and the GPA. However, broiler chickens fed the *S. boulardii* RC009 showed a greater surface area of absorption and lower villus height (9). These results partially agree with Roy and Ray (26), who observed an improvement in gut morphology in broilers, with increased villi height in the duodenum, jejunum, and ileum in feeding the chickens with 20 g *S. boulardii* (CNCM 1-1097) with a concentration of 2.0 x 10^{10} CFU/g per 100 kg feed. Besides, Jha *et al.* (30) and Poloni *et al.* (31) demonstrated the beneficial effect of probiotics on villus height, crypt depth, and a higher villus height-crypt depth ratio, indicating increased nutrient absorption by increasing the available surface area for nutrient uptake.

Establishing and sustaining a balanced microbiota within the avian GIT is crucial, as it plays a key role in suppressing pathogenic bacteria. The incorporation of probiotics into broiler diets has been shown to enhance productive performance by modulating gut microbial populations and optimizing nutrient absorption both quantitatively and qualitatively (30). Under certain conditions, probiotics may also serve as a viable alternative to subtherapeutic antibiotics. In this work, microbial diversity was similar in both groups and exhibited a notable increase in *Bacteroidetes* and *Firmicutes*. In this sense, the fact that few changes in richness were observed could indicate that the probiotic, with the improvements it produces in the animals, does not modify microbial diversity, which is positive from the perspective of the stability of the intestinal ecosystem (27). Other studies have shown no significant changes in microbiome diversity between the control and probiotic groups, particularly when *Bacillus subtilis* was administered (32). Han *et al.* (33) also found no change in microbial diversity with *Enterococcus faecalis* treatment group, and Trela *et al.* (34) observed significant differences only in fecal samples from the jejunum. Similarly, Gao *et al.* (35) found comparable levels of *Firmicutes* and *Proteobacteria* in broilers across different groups, underscoring the potential for probiotics to modify the gut flora composition.

Analysis of key metabolic mechanisms revealed an optimization of intestinal redox balance. In this context, *S. boulardii* RC009 may be altering the intestinal environment

to promote hydrogen (H_2) production by fermentative bacteria. *Methanobrevibacter*, a methanogenic archaeon, utilizes H_2 and CO_2 to generate methane via the reaction: $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$. By removing H_2, these microorganisms help maintain a low hydrogen potential in the gut, thereby favoring more energy-efficient fermentation pathways for the host (36,37).

Another key metabolic mechanism was the enhancement of energy efficiency. The removal of H_2 by *Methanobrevibacter* allowed fermentative bacteria to produce greater amounts of short-chain fatty acids (SCFAs) per molecule of fermented substrate. These SCFAs—primarily acetate, propionate, and butyrate—are absorbed by the intestinal epithelium and utilized as an energy source by the chicken (37).

Blake *et al.* (38) demonstrated that methanogens, such as *Methanobrevibacter smithii*, consume H_2 and CO_2 to produce methane, thereby reducing the partial pressure of hydrogen in the intestine. This reduction favors more efficient fermentative pathways, leading to increased production and absorption of short-chain fatty acids such as propionate, acetate, and butyrate, ultimately enhancing the host's metabolizable energy. Considering specific microbial interactions, a synergistic effect was observed with *S. boulardii*, which produces enzymes capable of degrading certain complex carbohydrates, thereby releasing substrates that promote hydrogen (H_2) production by fermentative bacteria (36). This additional H_2 creates an optimal environment for the growth of *Methanobrevibacter*, establishing an indirect symbiotic relationship. The observed reduction in bacterial families such as *Ruminococcaceae* and *Lachnospiraceae* may be attributed to *S. boulardii* altering substrate availability, favoring *Methanobrevibacter* over these competing bacteria (36).

Regarding the positive outcomes in productive parameters, it can be inferred that the dominant presence of *Methanobrevibacter* may be enhancing nutrient utilization by optimizing intestinal fermentation, thereby allowing greater energy extraction from the feed (36). This could explain why the chickens reached their target weight two days earlier. A microbiota with similar diversity but greater specialization—dominated by *Methanobrevibacter*—could be more efficient in converting feed into growth. In this context, a more specialized and metabolically efficient microbiota may contribute to improved poultry production. Some studies suggest that methanogens may modulate the intestinal immune response, indicating a potential anti-inflammatory effect (37). Reduced intestinal inflammation could lead to improved nutrient absorption and more efficient energy utilization for growth (37).

Conclusion

This study demonstrated that *Saccharomyces boulardii* RC009 preserved microbial diversity while promoting beneficial taxa such as *Methanobrevibacter*. This modulation enhanced digestibility through redox balance optimization and improved productive performance via increased short-chain fatty acid production, with a potential anti-inflammatory effect. These findings support the use of *S. boulardii* RC009 as a sustainable alternative to GPA in poultry production. Despite these findings, it is crucial to note that the applicability is limited to the specific strain and dose evaluated in this study. For a complete extrapolation of the mechanisms, it is necessary to investigate the direct metabolic function using metabolomics analysis to confirm the proposed mechanisms of action beyond taxonomic inference.

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Conflict of interest

The authors declare no conflicts of interest.

References

1. Zhao H, Comer L, Akram MZ, Corion M, Li Y, Everaert N. Recent advances in the application of microbiota transplantation in chickens. J Anim Sci Biotechnol. 2025;16:91. DOI: 10.1186/s40104-025-01233-6
2. Fathima S, Shanmugasundaram R, Adams D, Selvaraj RK. Gastrointestinal microbiota and their manipulation for improved growth and performance in chickens. Foods. 2022;11(10):1401. DOI: 10.3390/foods11101401
3. Zhou H, Yang L, Ding J, Xu K, Liu J, Zhu W, Zhu J, He C, Han C, Qin C, Luo H, Chen K, Zheng Y, Honaker CF, Zhang Y, Siegel PB, Meng H. Dynamics of small non-coding RNA profiles and the intestinal microbiome of high and low weight chickens. Front Microbiol. 2022;13:916280. DOI: 10.3389/fmicb.2022.916280
4. Okey SN. Alternative feed additives to antibiotics in improving health and performance in poultry and for the prevention of antimicrobial resistance: a review. Niger J Anim Sci Technol. 2023;6(1):65-76. [available at]
5. Halder N, Sunder J, De AK, Bhattacharya D, Joardar SN. Probiotics in poultry: a comprehensive review. J Basic Appl Zool. 2024;85:23. DOI: 10.1186/s41936-024-00379-5
6. Ogbuewu IP, Mabelebele M, Sebola NA, Mbajiorgu C. Bacillus probiotics as alternatives to in-feed antibiotics and its influence on growth, serum chemistry, antioxidant status, intestinal histomorphology, and lesion scores in disease-challenged broiler chickens. Front Vet Sci. 2022;9:876725. DOI: 10.3389/fvets.2022.876725
7. Tomczyk G, Niczyporuk JS, Kozdruń W, Sawicka-Durkalec A, Bocian Ł, Barabasz M, Michalski M. Probiotic supplementation as an alternative to antibiotics in broiler chickens. J Vet Res. 2024;68(1):147-154. DOI: 10.2478/jvetres-2024-0009

8. Food and Agriculture Organization of the United Nations, World Health Organization. Probiotics in food: health and nutritional properties and guidelines for evaluation. Rome: FAO; 2006. [available at]
9. Yue T, Lu Y, Ding W, Xu B, Zhang C, Li L, Jian F, Huang S. The role of probiotics, prebiotics, synbiotics, and postbiotics in livestock and poultry gut health: a review. Metabolites. 2025;15(7):478. DOI: 10.3390/metabo15070478
10. Nath SK, Hossain MT, Ferdous M, Siddika MA, Hossain A, Maruf AA, Chowdhory AT, Nath TC. Effects of antibiotic, acidifier, and probiotic supplementation on mortality rates, lipoprotein profile, and carcass traits of broiler chickens. Vet Anim Sci. 2023;22:100325. DOI: 10.1016/j.vas.2023.100325
11. Mirsalami SM, Mirsalami M. Effects of duo-strain probiotics on growth, digestion, and gut health in broiler chickens. Vet Anim Sci. 2024;24:100343. DOI: 10.1016/j.vas.2024.100343
12. Fochesato AS, Martínez MP, Cuello D, Poloni VL, Luna MJ, Magnoli AP, Fernández C, Cavaglieri LR. Effects of a mixed additive based on Saccharomyces cerevisiae and Lactobacillus rhamnosus on broilers exposed to aflatoxin B1 by contaminated feed. Rev Argent Microbiol. 2024;56(3):312-321. DOI: 10.1016/j.ram.2023.11.006
13. Magnoli AP, Parada J, Luna MJ, Corti M, Escobar FM, Fernández C, Coniglio MV, Ortiz ME, Wittouck P, Watson S, Cristofolini LA, Cavaglieri L. Impact of probiotic Saccharomyces cerevisiae var. boulardii RC009 alone and in combination with a phytase in broiler chickens fed with antibiotic-free diets. Adv Res Biol Sci. 2024;16:1-10. DOI: 10.47278/journal.abr/2024.006
14. Parada J, Magnoli A, Isgro MC, Poloni V, Fochesato A, Martínez MP, Carranza A, Cavaglieri L. In-feed nutritional additive probiotic Saccharomyces boulardii RC009 can substitute for prophylactic antibiotics and improve the production and health of weaning pigs. Vet World. 2023;16(5):1035-1042. DOI: 10.14202/vetworld.2023.1035-1042
15. Parada J, Magnoli A, Poloni V, Corti Isgro M, Rosales Cavaglieri L, Luna MJ, Carranza A, Cavaglieri L. Pediococcus pentosaceus RC007 and Saccharomyces boulardii RC009 as antibiotic alternatives for gut health in post-weaning pigs. J Appl Microbiol. 2024;135(11):lxae282. DOI: 10.1093/jambio/lxae282
16. Cobb-Vantress. Cobb breeder management guide. Arkansas: Cobb-Vantress Inc; 2024. [available at]
17. Poloni VL, Magnoli AP, Fochesato A, Poloni L, Cristofolini A, Merkis C, Schifferli Riquelme C, Schifferli Maldonado F, Montenegro M, Cavaglieri L. Probiotic gut-borne Saccharomyces cerevisiae reduces liver toxicity caused by aflatoxins in weanling piglets. World Mycotoxin J. 2021;14(3):1-10. DOI: 10.3920/WMJ2020.2629
18. Luna MJ, Isgro MC, Cavaglieri LAR, Coniglio MV, Ortiz ME, Cavaglieri LR, Parada J, Magnoli AP. Experimental evaluation of the effects of commercial additive (plant extracts) as an alternative to growth-promoting antibiotics in broiler chickens. Vet World. 2025;18(3):636-645. DOI: 10.14202/vetworld.2025.636-645
19. Sohail MU, Hume ME, Byrd JA, Nisbet DJ, Ijaz A, Sohail A, Shabbir MZ, Rehman H. Effect of supplementation of prebiotic mannan-oligosaccharides and probiotic mixture on growth performance of broilers subjected to chronic heat stress. Poult Sci. 2012;91(9):2235-2240. DOI: 10.3382/ps.2012-02182
20. Magoc T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011;27(21):2957-2963. DOI: 10.1093/bioinformatics/btr507
21. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R, Mills DA, Caporaso JG. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods. 2013;10(1):57-59. DOI: 10.1038/nmeth.2276
22. Edgar RC, Haas BJ, Clemente JC, Quince C, Knight R. UCHIME improves sensitivity and speed of chimera detection. Bioinformatics. 2011;27(16):2194-2200. DOI: 10.1093/bioinformatics/btr381
23. Wickham H. ggplot2: elegant graphics for data analysis. USA: Springer; 2016. 260 p. DOI: 10.1007/978-3-319-24277-4
24. Quinn GP, Keough MJ. Experimental design and data analysis for biologists. UK: Cambridge University Press; 2002. DOI: 10.1017/CBO9780511806384
25. Di Rienzo JA, Casanoves F, Balzarini MG, Gonzalez L, Tablada M, Robledo CW. InfoStat version 2020. Argentina: Universidad Nacional de Córdoba; 2020. [available at]
26. Roy BC, Ray BC. Potentiality of Saccharomyces cerevisiae in replacing antibiotic growth promoters on growth, gut microbiology, histology, and serum antibody titers of commercial broilers. J Appl Poult Res. 2023;32(3):100352. DOI: 10.1016/j.japr.2023.100352
27. Lin J, Comi M, Vera P, Alessandro A, Qiu K, Wang J, Wu SG, Qi GH, Zhang HJ. Effects of Saccharomyces cerevisiae hydrolysate on growth performance, immunity function, and intestinal health in broilers. Poult Sci. 2023;102:102237. DOI: 10.1016/j.psj.2022.102237
28. Parada J, Magnoli AP, Alonso V, Díaz Vergara L, Corti Isgro M, Posse JJT, Montenegro MA, Cavaglieri L. Inclusion of *Saccharomyces cerevisiae* var. *boulardii* RC009 and *Pediococcus pentosaceus* RC007 as a probiotic additive in pigs' postweaning diets and its effect on meat composition, carcass characteristics, and fatty acids profile after slaughter. Vet Med Int. 2024;2024:6658120. DOI: 10.1155/2024/6658120
29. Markova K, Kreisinger J, Vinkler M. Are there consistent effects of gut microbiota composition on performance, productivity and condition in poultry?. Poult Sci. 2024;103:103752. DOI: 10.1016/j.psj.2024.103752
30. Jha R, Das R, Oak S, Mishra P. Probiotics (direct-fed microbials) in poultry nutrition and their effects on nutrient utilization, growth and laying performance, and gut health: a systematic review. Animals. 2020;10(10):1863. DOI: 10.3390/ani10101863
31. Poloni VL, Magnoli AP, Fochesato A, Cristofolini A, Caverzan M, Merkis C, Montenegro M, Cavaglieri LA. *Saccharomyces cerevisiae* RC016-based feed additive reduces liver toxicity, residual aflatoxin B1 levels and positively influences intestinal morphology in broiler chickens fed chronic aflatoxin B1-contaminated diets. Anim Nutr. 2020;6(1):31-38. DOI: 10.1016/j.aninu.2019.11.006
32. Ma Y, Wang W, Zhang H, Wang J, Zhang W, Gao J, Wu S, Qi G. Supplemental Bacillus subtilis DSM 32315 manipulates intestinal structure and microbial composition in broiler chickens. Sci Rep. 2018;8(1):15358. DOI: 10.1038/s41598-018-33762-8
33. Han W, Zhang XL, Wang DW, Li LY, Liu GL, Li AK, Zhao YX. Effects of microencapsulated Enterococcus faecalis CG1.0007 on growth performance, antioxidation activity, and intestinal microbiota in broiler chickens. J Anim Sci. 2013;91(9):4374-4382. DOI: 10.2527/jas.2012-5956
34. Trela J, Kieronczyk B, Hautekiet V, Józefiak D. Combination of Bacillus licheniformis and salinomycin: effect on the growth performance and GIT microbial populations of broiler chickens. Animals. 2020;10(5):889. DOI: 10.3390/ani10050889
35. Gao P, Hou Q, Kwok LY, Huo D, Feng S, Zhang H. Effect of feeding Lactobacillus plantarum P-8 on the faecal microbiota of broiler chickens exposed to lincomycin. Sci Bull. 2017;62(2):105-113. DOI: 10.1016/j.scib.2017.01.001
36. Misiukiewicz A, Gao M, Filipiak W, Cieslak A, Patra AK, Szumacher-Strabel M. Methanogens and methane production in the digestive systems of nonruminant farm animals. Animal. 2021;15:100060. DOI: 10.1016/j.animal.2020.100060
37. Zengin OD, Aydin S. The hidden influence of methanogens in the gut microbiota. London: IntechOpen; 2025. DOI: 10.5772/intechopen.1008814
38. Blake D, Taylor LD, Leclercq S, Detry R, et al. Methanogenesis associated with altered microbial production of short-chain fatty acids and host metabolizable energy. ISME J. 2025;19:wraf103. DOI: 10.1093/ismejo/wraf103

Saccharomyces boulardii RC009 كبديل للمضادات الحيوية الوقائية: التأثيرات على دجاج التسمين والأداء وميكروبيوم الأمعاء

أليخاندرا باولا ماغنولي، مار إرما جوليتا لونا، لوريانو خوسيه جيوردانو، مايتي كورتي إيسغرو، فاليريا لورينا بولوني، دانيلو غييرمو تشيشين، جوليان بارادا، ليليا رين-كافاجليري

[1]قسم الإنتاج الحيواني من جامعة ريو كوارتو، قرطبة، [2]مجلس البحوث الوطنية العلمية والتقنية، بوينس آيرس، [3]مركز أبحاث الطب الانتقالي، أموشاستكيو، مجلس البحوث الوطنية العلمية والتقنية، قرطبة، [4]قسم علم أمراض الحيوان [5]قسم الأحياء الدقيقة والمناعة، جامعة ريو كوارتو الوطنية، قرطبة، [6]جامعة قرطبة للعلوم الطبية الحيوية، قرطبة، الأرجنتين

الخلاصة

تقيم هذه الدراسة آثار تكملة علف الدجاج اللاحم مع بروبيوتيك Saccharomyces cerevisiae var. boulardii RC009 كبديل مستدام للمضادات الحيوية الوقائية. كان التركيز على الأداء الإنتاجي ، وتكوين ميكروبيوم الأمعاء ، ووقت الوصول إلى وزن الذبح. تضمنت المحاكمة ٧٧٢٠٠ دجاجة كوب-٥٠٠ دجاجة عمرها يوم واحد موضوعة في ٥ أقلام أرضية. تمت مقارنة مجموعتين: التحكم (النظام الغذائي القاعدي + المضادات الحيوية) والبروبيوتيك (النظام الغذائي القاعدي + *S. boulardii* RC009، بدون مضادات حيوية). تم إعطاء البروبيوتيك من اليوم ٠ حتى وصلت الطيور إلى ٣ كجم. أظهرت مجموعة البروبيوتيك تحسينات ملحوظة: وصل الذكور إلى وزن ذبح أعلى بنسبة ١٠,٩٧ ٪ وحققوا حجم السوق قبل يومين من الضوابط. أشار التحليل النسيجي إلى صحة الأمعاء المحفوظة ، مع عدم وجود فروق ذات دلالة إحصائية بين المجموعات. كشف 16S rDNA أن البروبيوتيك غير بشكل كبير ميكروبيوتا الأمعاء. على الرغم من انخفاض التنوع الميكروبي الكلي بشكل طفيف، إلا أنه كان هناك تخصيب ملحوظ للعتائق الميثانوجينية، ولا سيما *Methanobrevibacter* والـ *Methanobacteriales* الأخرى، إلى جانب انخفاض في العائلات البكتيرية السائدة مثل *Ruminococcaceae* و *Lachnospiraceae*. هذه النتائج تشير إلى أن مكملات مع *S. boulardii* RC009 تحسين أداء اللاحم عن طريق زيادة وزن الذبح وتقليل الوقت إلى السوق. يشير الارتفاع في العتائق الميثانوجينية إلى تعزيز توازن الأكسدة والاختزال والزيادات المحتملة في إنتاج الأحماض الدهنية قصيرة السلسلة، مما يساهم في تحسين قابلية الهضم والتأثيرات المضادة للالتهابات. تدعم هذه النتائج *S. boulardii* RC009 كبديل قابل للتطبيق ومستدام لمحفزات نمو المضادات الحيوية في تربية الدواجن.