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IMPACT OF PROBIOTIC SACCHAROMYCES CEREVISIAE VAR. BOULARDII RC009 ALONE AND IN COMBINATION WITH A PHYTASE IN BROILER CHICKENS FED WITH ANTIBIOTIC-FREE DIETS

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ABSTRACT

The aim of the study was to assess the impact of the *Saccharomyces boulardii* RC009 alone and in combination with a phytase on productive performance, biochemistry, apparent ileal phosphorus digestibility, genotoxicity and histomorphometric parameters in replacement of growth-promoting antibiotics. Two hundred and four 1-day-old male broiler chickens were weighed and redistributed in 3 replicates per treatment with 17 broilers chickens each. Throughout the 49-day experimental period, the broiler chickens were provided with both starter and finisher diets corresponding to each treatment. Treatments (T) were T1: basal diet (BD - control with AGP); T2: BD (without AGP) + *S. boulardii* RC009 (200g/T, 1 x 10^{12} CFU/T feed); T3: BD (without AGP) + *S. boulardii* RC009) + phytase (1000 FTU/T); T4: BD (without AGP) + phytase. The results showed that all treatments were able to improve the productive parameters studied such as DWG and DFI ($P \leq 0.05$) when compared to the control. The T3 had the highest value followed by T4 and T2. The best value of CI was obtained for T2 followed by T3 and T4. There is no effect of the probiotic or the enzyme alone or in combination on the biochemical parameters evaluated. The treatment T3 improved the weight of leg-thigh and poultry breast ($P \leq 0.05$). The digestibility of phosphorus showed significant differences between treatments ($P \leq 0.05$). The histomorphometric parameters were significantly influenced, impacting both the radio and absorptive surface areas, T3 had the best absorptive surface area. The frequency of micronucleus in bone marrow cells of broiler chickens was not affected by any of the studied treatments. The utilization of *S. boulardii* RC009 alone or combined with phytase notably enhanced productivity parameters, economically significant carcass weight, and histomorphometric characteristics in the small intestine. Moreover, they did not exert toxicity. These results suggest their promising potential for use either independently or in combination as substitutes for antibiotic growth promoters.

Keywords: Probiotic *S. boulardii* RC009, Phytase, Antibiotic growth promoters, Broiler chickens, Production parameters

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1. INTRODUCTION

An optimal absorption of nutrients allows an efficient conversion which is essential for the production and welfare of animals. For several decades, antibiotics addition has been used as growth promoters to maintain intestinal health and improve digestive efficiency (Abd El-Hack et al. 2022). Growing concern surrounds the detrimental effects of indiscriminate antibiotic administration in animals and its potential impact on human health, particularly regarding the transmission of resistance-inducing genes to the human microbiota. This led the European Community to ban the use of growth promoter antibiotics (Regulation EC N°1831/2006). Probiotics are suggested as a viable alternative to antibiotics for promoting growth. They stimulate a balanced gut microbiota, supporting intestinal integrity and functionality of the digestive mucosa (Anadón et al. 2019; Rashid et al. 2023).

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The inclusion of probiotics in poultry production improves the nutritional needs of birds, optimizing of the productive efficiency. Nevertheless, achieving this objective is inherently tied to ensuring the good health and proper welfare of the birds (Rinttilä and Apajalahti 2013). More than 2000 years ago, Hippocrates already pointed out that " the totality of the diseases originate in the intestine". According to Pluske et al. (2018) the gut health encompasses various components such the optimal digestion and absorption of nutrients, a diverse and stable microbiota, an effective intestinal immune system, a strong intestinal barrier against pathogens and toxins, as well as a competent neuroendocrine system.

The addition of probiotics to poultry diets is of special relevance today. Probiotics are defined as "live microorganisms that, when administered in adequate amounts, are beneficial to the host 's health. In poultry, maintaining a stable intestinal biota is crucial to prevent dysbiosis, which can predispose birds to infectious diseases (Dowarah et al. 2017; Kiros et al. 2018) together with great economic losses in the sector. The utilization of bacteria and yeasts as probiotics has shown effectiveness in promoting the growth of beneficial intestinal microflora and some in acting as mycotoxin adsorbers (Magnoli et al. 2016, 2017, 2018; Anadón et al. 2019; Poloni et al. 2020; Coniglio et al. 2023a,b). Probiotics help in maintaining a balance of intestinal biota promoting proper health and a productive performance best (Alagawany et al. 2021). The more important mode of action for most probiotics involves reducing gut pH by to the production of volatile fatty acid and organic acids due to your metabolism. (Al-Fatah 2020), thus they could decrease the growth of pathogenic bacteria such as *Salmonella* and *E. coli* strains (Swelum et al. 2021). Probiotics have also shown improve gut development, related with to a larger surface area for absorbing nutrients (Abd El-Hack et al. 2022).

The use and development of enzymatic compounds for feeding birds in their different physiological and productive stages, represents a great opportunity to increase production. Exogenous enzymes enhance the nutritional value of food, expanding the potential use of raw materials. This offers greater flexibility for food plants and increased profits for producers by boosting poultry production (Alagawany et al. 2018). The combined use of probiotics alongside enzymes, coccidiostats, phytobiotics, and other additives, coupled with effective management practices and robust biosecurity programs, has proven to be a possible option to replace the growth-promoting antibiotics (Mehdi et al. 2018; Ismael et al. 2022).

The aim of the study was to assess the impact of the *S. boulardii* RC009 alone and in combination with a phytase enzyme on productive performance, biochemistry (glucose, cholesterol, calcium and phosphorus), apparent ileal digestibility of phosphorus and histomorphometric parameters in replacement of growth promoting antibiotics. Moreover, the genotoxicity of the treatments was studied.

2. MATERIALS AND METHODS

The animal care and use committee of the National University of Río Cuarto, permitted the research procedure that was carried out in the current study.

2.1. Probiotic Additive

The probiotic additive used in the current experiment was acquired from a Collection of Industrial Microbiology, Biotechnology Applied to Animal Feed Additives group (BIOAPLA) of the National University of Río Cuarto. This product is composed by *Saccharomyces cerevisiae var. boulardii* RC009 (*S. boulardii*) (Armando et al. 2011). The concentration of *S. boulardii* RC009 was $1x10^{10}$ CFU/g (Fochesato et al. 2028; Poloni et al. 2020). The probiotic additive (200g) was mixed with the corresponding diet to reach $1x10^{12}$ CFU/T of feed.

2.2. Design and animal management

Two hundred and four male chicks, one-day old (Commercial line Arbor Acres) were obtained from a commercial hatchery. These chicks were feed with a standard maize-soybean meal starter and finisher commercial diet (basal diet) with and without AGP (Avilamycin 10) (Table 1), the formulation of experimental diets, and the animal management were realized following the methodology described by Magnoli et al (2021). Broiler chicks of eight days old were weighed individually (130.01g±6.88) and redistributed in 3 replicates per treatment with 17 broilers chickens. The experimental design consisting of four treatments is presented in Table 2, during a period the 45 days.

2.3. Parameters Evaluated

2.3.1. Productive Parameters: The broilers' weights were recorded at the beginning, on a weekly basis, and at the conclusion of the study. Morbidity and mortality were recorded every day. The evaluated productive parameters were daily weight gain (DWG-g) calculated by the difference between final and initial weight dividing the weight by the number of assay days, the amount of feed left in the feeder was weighed and the difference was divided by the number of assay days to estimate daily feed intake (DFI-g), and conversion index (CI), ratio between DFI and DWG (daily feed intake: daily weight gain), were determined for each treatment from day 1 to day 49 of the assay.

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Table 1: Experimental diet composition on a fed basis (g/kg)

Items	Diets	
	Starter	Finisher
Yellow corn	629.0	672
Soybean flour	226.0	190.0
Heat treated soybeans	55.0	50.0
Meat meal 40%	69.0	70.0
Mix of vitamins and minerals[1]	1.50	1.5
NaCl	2.00	2.0
Calcite 38%	3.50	3.0
sunflower oil	10.0	10.0
DL-Methionine	1.6	1.0
L-Lysine	1.0	—
Monensin	0.5	0.5
Total	1,000.0	1,000.0
Proximal Composition (g/kg diet)		
Crude protein	203.3	189.0
crude fat	54.7	55.3
Crude fibre	33.4	30.8
Calcium	9.7	9.5
Total phosphorus	5.9	5.7
Lysine	11.4	9.3
Methionine	5.0	4.2
Tryptophan	2.4	2.2
ME, kcal.kg	3,047.0	3,062.0

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

2.3.2. Biochemical Parameters: At 45 days the feeding assay was concluded, 6 broiler chickens per replicate of each and blood samples of 5mL without anticoagulant were collected from subclavical vein. The samples were immediately remitted to the laboratory to evaluate biochemical parameters such as cholesterol, glucose, calcium and phosphorus. These concentrations were determined with a clinical chemistry analyzer according to the manufacturer's recommended procedure (Wiener Laboratory, 2000).

The following reagents were used: cholesterol: Lipid AA Enzyme Cholestat; Phosphorus: phosphatemia UV AA; Calcium: Ca-Colour Arsenazo III AA; Glucose: enzymatic glycemia AA. The serum biochemical values were grouped and expressed as mean±pooled SEM.

Table 2: Experimental plan

Treatment	Basal Diet with AGP (Avilamycin 100g/T) or without AGP	*S. boulardii* RC009 (200g/T, 1×10^{12} CFU/T of feed)	Phytase (1000 (FTU/T of feed)
T1 (Control)	Basal diet +AGP	No	No
T2	Basal diet	Yes	No
T3	Basal diet	Yes	Yes
T4	Basal diet	No	Yes

2.3.3. Weight of the Carcass Cuts: At 45 days the feeding assay was finished, 18 broiler chickens per treatment were randomly chosen and killed by cervical dislocation. Then, euthanasia was performed by white bloodletting as recommended by the UNRC ethics committee and a detailed necropsy of the birds was carried out. The weight of the most economically important carcass cuts (leg, thigh and breast) was determined.

2.3.4. Apparent Ileal Digestibility of phosphorus: the indicator method was used for its determination, for this, chromium oxide (250g, ANEDRA) was added to the finisher diet as an indigestibility marker (2g/kg of food) during 5 days before the sacrifice. At the end of the assay, 6 broilers per replicate (49 days old) were randomly selected and sacrificed, then the ileal content was sampled refrigerated (-20°C). The samples were lyophilized, and the concentration of chromium oxide and phosphorus was determined from 100g of sample by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) according to Kavanagh et al. (2001). The apparent digestibility coefficient of phosphorus was calculated using the following equations:

ADC: [1 - PCID*CID/CPD*CIID] *100

Where ADC: apparent digestibility coefficient; PCID: Phosphorus concentration in the ileal digesta; CID: Concentration of indicator in the diets; CPD: Concentration of phosphorus in the diets; CIID: Concentration of indicator in the ileal digesta.

2.3.5. Histomorphometric Parameters: The tissue samples for histology were taken from duodenum and were processed following the methodology described by Poloni et al. (2021). The morphometric measurements taken from the intestinal histological sections (length, width of villus and intestinal crypt depth) was estimated according to Nain et al. (2012).

2.3.5.1. Apparent Absorptive Area: The absorptive surface area of the duodenal villus was estimated by considering a villus as a cylindrical structure (Nain et al. 2012). Villus absorptive surface area was calculated using the following formula according to Sohail et al. (2012): Villus absorptive surface area = $2\pi \times$ (average villus width/2) $\times$ villus height.

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2.3.6. Genotoxicity Assay of *S. boulardii* RC009 and phytase: was evaluated in broiler chicken's erythrocytes according to Magnoli et al. (2021). To establish the genotoxic capacity of the *S. boulardii* RC009 and phytase, we determined the number of micronucleus erythrocytes (EMN) in 1000 polychromatic erythrocytes (PCE) per broiler chicken. The slides were scored blindly using a light microscope at a 1000 x magnification.

2.4. Statistical Analysis

Data were analyzed by a general linear mixed model (GLMM) (version 2.03; Córdoba, Argentina). Data were analyzed by analysis of variance (ANOVA). Means were compared using Fisher's protected least significant test (LSD) ($P<0.05$).

3. RESULTS

3.1. Productive Parameters

The results obtained from the productive parameters of broiler chickens fed with different diets are shown in Table 3. The treatment *S. boulardii* RC009 plus phytase was able to significantly improve the productive parameters studied, such as DWG, DFI and CI ($P\leq0.05$). The daily weight gain was higher in the treatment T3 (*S. boulardii* RC009 plus phytase) (201g±99), also, the DFI was the highest compared to the other treatments (354g±65). The animals fed with *S. boulardii* RC009 alone and phytase alone were also able to significantly improve the productive parameters studied, when contrasted with treatment T1 ($P\leq0.05$). The values of the productive parameters obtained with *S. boulardii* RC009 without the addition of APC to the diet, are within those expected for the tested line. The CI value was the lowest in the animals fed with *S. boulardii* RC009 alone (1.86).

3.2. Biochemical Parameters

Table 4 shows the results obtained from the biochemical parameters of broiler chickens. The values of the biochemical parameters were within normal values. The glucose, cholesterol, calcium and phosphorus values there were no differences ($P\leq0.05$).

Table 3: Productive parameters in broiler chickens obtained with *S. boulardii* RC009 and phytase enzyme alone and in mixture

Treatments	Productive parameters		
	DWG (g)	DFI (g)	CI
Control	143±26a	288±68a	2.02
S. boulardii RC009	153±26b	285±103a	1.86
S. boulardii RC009 + phytase	201±99c	354±65b	1.76
Phytase	154±24b	284±54a	1.84

DWG: daily weight gain; DFI: daily feed intake; CI: conversion index. Values (mean±SD) in the same row with different superscripts indicate tended to differ or differ significantly ($P<0.05$).

Table 4: Biochemical parameters (mg/dL) in broiler chickens obtained with *S. boulardii* RC009 and phytase enzyme alone and in mixture

Treatments	Biochemical Parameters			
	Glucose	Chol	Ca	Phos
Control	178.3±20.7	128.5±17.3	7.3±3.8	8.70±2.6
S. boulardii RC009	170.5±8.5	136.2±10.7	8.4±2.0	8.42±1.0
S. boulardii RC009 + phytase	172.2±13.9	134.5±14.8	8.8±1.6	8.36±2.3
Phytase	179.1±5.5	134.2±3.14	9.2±0.2	8.40±0.7

Values (Mean±SD) with same superscripts indicate not tend to differ ($P\leq0.05$). Chol=Cholesterol; Ca=Calcium; Phos=Phosphorus.

3.3. Weight of Carcass Cuts

Table 5 shows the weight of economically important cuts of the broiler chickens at 45 d. *Saccharomyces boulardii* RC009 alone, *S. boulardii* RC009 plus phytase and phytase alone showed a significant increase in the weight of both, leg-thigh and breast ($P\leq0.05$). The *S. boulardii* RC009 plus phytase exerted a synergistic and significantly greater effect on the weight of the mentioned cuts.

3.4. Apparent Ileal Digestibility

The obtained results depict the apparent ileal phosphorus digestibility of broiler chickens fed with different diets are shown in Table 6. Apparent ileal phosphorus digestibility values showed effects whit the addition of the *S. boulardii* RC009 and/or phytase ($P\leq0.05$). The animals fed with *S. boulardii* RC009 plus phytase showed the highest values of phosphorus digestibility (75.8±1.2), followed by the animals fed with the phytase alone (72.3±1.1), and *S. boulardii* RC009 alone (69.2±1.1), compared to the control (42.5±0.6).

3.4. Histomorphometric Parameters

3.4.1. Apparent Absorptive Area

In the Table 7 is present the parameters histomorphometric for the different treatments. There was difference for the height and width of the intestinal villi values and crypt depth whit the addition of the *S. boulardii* RC009 and/or phytase ($P\leq0.05$). The treatments 2, 3 and 4 showed duodenal villi significantly higher in comparison of the treatment 1 ($P\leq0.05$); treatment 3 (*S. boulardii* RC009 plus phytase) showed the highest values of villi height (919.58µm) (Fig. 1). The highest values of villi width were for treatment 2 (*S. boulardii* RC009 alone) (231.38µm) followed by treatment 3 (*S. boulardii* RC009 plus phytase) (222.16µm). Similar behavior was shown regarding the crypt depth. The ratio of villus to crypt did not show significant differences among treatments ($P\geq0.05$). The

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apparent absorption area exhibited significant differences among treatments when compared to the control; the largest apparent adsorption area was obtained from *S. boulardii* RC009 plus phytase treatment (639.996,43µm) followed by *S. boulardii* RC009 alone treatment.

Table 5: Effect of *S. boulardii* RC009 and phytase enzyme alone and in mixture on the weight of economic important cuts from broiler chickens

Treatments	Leg-thigh (g)	Poultry Breast (g)
Control	368.2±33.0a	506.8±62.9a
S. boulardii RC009	474.7±44.4b	630.5±66.8b
S. boulardii RC009 + phytase	573.0±65.3c	778.5±129.0c
phytase	515±125.2bc	750.0±109.7bc

Values (Mean±SD) differ significantly(P≤0.05) bearing different alphabets in a column.

Table 6: Influence of *S. boulardii* RC009 and phytase enzyme alone and in mixture on the apparent ileal phosphorus digestibility in broiler chickens

Treatments	Phos in ileal digesta (mg/kg)	ileal Phos digestibility (%)
Control	689.4±137.8	42.5±0.6d
S. boulardii RC009	711.9±142.4	69.2±1.1c
S. boulardii RC009 + phytase	808.4±161.7	75.8±1.2a
phytase	860.7±172.1	72.3±1.1b

Values (Mean±SD) differ significantly(P≤0.05) bearing different alphabets in a column. Phosphorus (Phos) in diet was 519.35±101.31mg/kg.

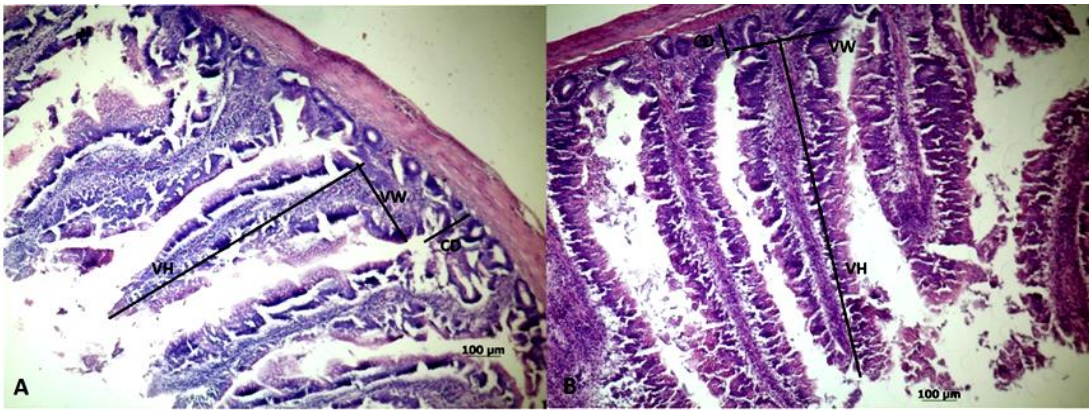

Fig. 1: Histological representation of duodenal mucosa of control and probiotic plus phytase treated in broilers chicken. The lines represent villus height, and crypt depth respectively VH: villus height; CD: crypt depth. **A:** control (T1); **B:** *S. boulardii* RC009 + phytase (T3). Scale bar = 100 µm. H & E stain.

Table 7: Influence of probiotic *S. boulardii* RC009 and phytase enzyme alone and in mixture on the histomorphometric parameters in poultry.

Treatments	Duodenum Villus (µm)				
	Height	Width	Crypt Depth	Ratio	Absorptive Surface Area
Control	790.21±148.34a	193.6±66.31a	96.25±26.01a	8.66±2.38a	490,047.94±211,256.03ab
S. boulardii RC009	802.80±238.97a	231.38±75.4b	119.82±27.19b	7.16±2.95a	550,314.72±160,398.27bc
S. boulardii RC009 + phytase	919.58±240.71b	222.16±48.82b	120.64±28.56b	8.54±5.74a	639,996.43±217,704.32c
Phytase	793.55±172.04a	167.02±54.36a	115.74±22.59b	7.25±2.72a	423,737.23±168,266.81a

Values (Mean+SD) differ significantly(P≤0.05) bearing different alphabets in the same column.

3.5. Genotoxicity Assay

No genotoxic effects were observed with the oral administration of *S. boulardii* RC009 and phytase. The number of micronucleus erythrocytes (EMN) in the control treatment was 1.73±0.38 (Fig. 2). The addition of *S. boulardii* RC009 and phytase did not significantly increase the number the EMN. Fig. 3 showed a typical micronucleus inside the erythrocyte is indicated with an arrow.

4. DISCUSSION

In this study, the influence of *S. boulardii* RC009, both alone and in combination with a phytase enzyme, on the productive performance, biochemistry, apparent ileal digestibility of phosphorus, and histomorphometric parameters was examined in broiler chickens as a substitute for growth-promoting antibiotics.

The use and development of enzymatic compounds for feeding birds in their different physiological and productive stages represents a great opportunity to increase production. Utilizing exogenous enzymes enhances the nutritional value of food, expanding the potential use of raw materials. This offers greater flexibility to food plants and increased profits to producers due to the productive increase of poultry (Velázquez-De Lucio et al. 2021).

In the present study the animals fed with 1000 FTU of phytase alone were able to improve the DWG. Also, an increase in the weight of leg-thigh and breast was demonstrated in broiler chickens fed with the *S. boulardii* RC009 plus phytase. Our results partially align with various studies by different authors, who demonstrated that productive parameters of broilers were not different with exogenous enzyme supplementation (Hanna et al. 2008; Cowieson and Ravindran 2008; Ding et al. 2016; Gul and Alayah 2023). Also, Dalólio et al. (2016) showed that the addition

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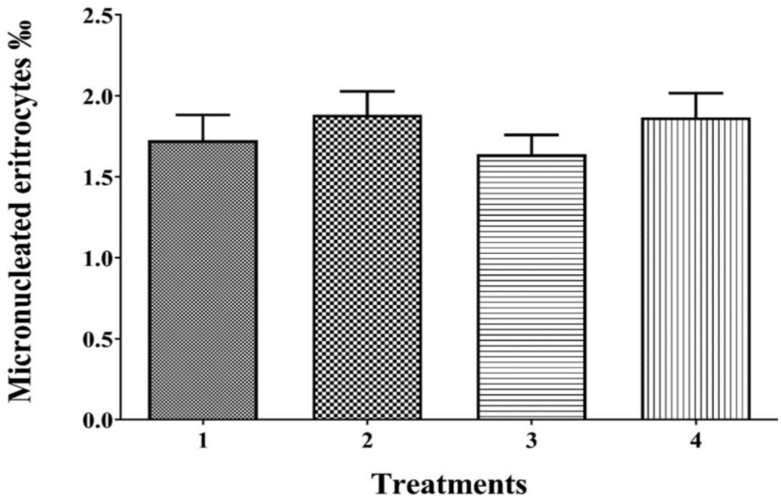

Fig. 2: Number of micronucleus erythrocytes (EMN) in 1000 polychromatic erythrocytes (PCE) per broiler chicken. Treatments (T) T1: basal diet (BD - control with AGP); T2: BD (without AGP) + *S. boulardii* RC009; T3: BD (without AGP) + *S. boulardii* RC009 + phytase; T4: BD (without AGP) + phytase. N= 6. Data (Mean ± SD) with same superscripts indicate not tend to differ (P≤0.05).

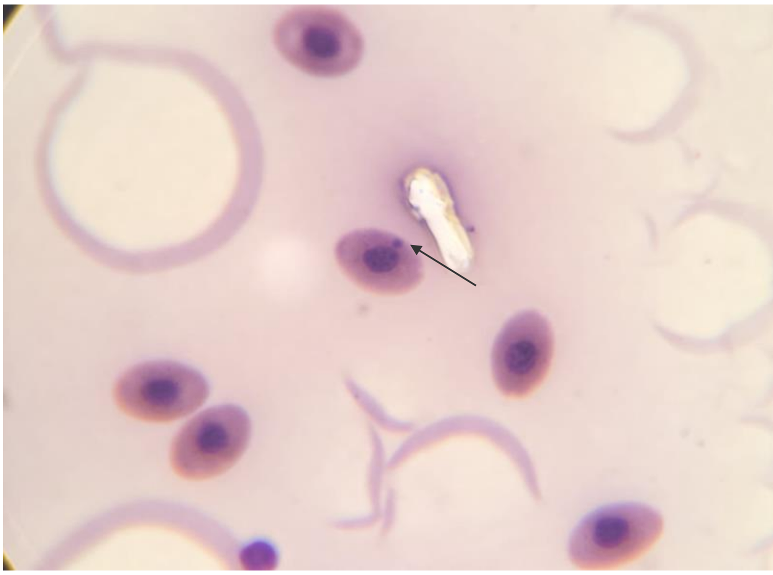

Fig. 3: Microphotograph (optical microscopical) of micronucleus indicated with an arrow, 20 x magnification. Stained with May Grunwald and Giemsa stains**.**

of enzyme complex (phytase, protease, xylanase, β-glucanase, cellulase, amylase, and pectinase) did not affect performance, carcass yield and meat quality, with the exception of the performance characteristics of the breast and the wings at 42 days of age in broiler chicks. Several studies have demonstrated a positive impact on the weight of economic important cuts of broilers fed with probiotic and enzyme (Midilli and Tuncer, 2001; Kaushal et al. 2019). In opposition, Hassan et al. (2011); Kiarie et al. (2014) and Flores et al. (2016) revealed positive effects on productive parameters in broilers feed with enzyme supplementation. In addition, Metwally et al. (2020) showed that the body weight of birds fed with 1500 FTU phytase was improved compared to the control groups.

The primary objective of incorporating exogenous phytase in diets is to enhance the utilization of accessible phosphorus and calcium found in cereal grains. Additionally, it aims to improve the use of other nutrients such as macrominerals, microminerals, amino acids, and proteins. Phytate is hydrolyzed to inositol and inorganic phosphate by phytase enzyme. Approximately 60% of the total phosphorus in cereal grains is found in phytate complexes, indigestible to birds. Phytate binds proteins, hydrolytic enzymes, fats, vitamins, and cations such as Cu, Zn, Ca, Fe, Mg, Mn, reducing significant in nutrient availability mentioned (Mahmood et al. 2018).

On other hand, the inclusion of *S. boulardii* RC009 alone in broilers resulted in an improvement in DWG, Additionally, it is essential to note also that CI value was the lowest. The results of this study relatively agree with Sen et al. (2012), who demonstrated a higher DWG, DFI and better CI in birds fed with 3.0 and 4.5g *B. subtilis* LS 1-2g/kg of diet, with 10^8 and 10^9 CFU/kg diet for 35 days. Also, Also, our results are in agreement with those of Mountzouris et al. (2007) who demonstrated an improvement performance productive in broilers fed with 1g/kg probiotic (Biomin Poultry5Star, BIOMIN) for 42 days compared to in broilers fed with avilamycin. In addition, El-Manawey et al. (2021) demonstrated an improved production performance that broiler chickens fed whole yeast of S. cerevisiae (0.1%) for 35 days. Analogous results were reported by Hana et al. (2015) who used 3.0g probiotic

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yeast product per kg diet in broiler chickens. Also, Shankar et al. (2017) reported that inclusion of 2.0g probiotic yeast (*S. cerevisiae*) per kg to broiler diets improved body weight gain and feed conversion ratio. Ogbuewu et al. (2020) confirmed that probiotic yeast of *S. cerevisiae* origin improved body weight gain, and feed conversion ratio while it reduced feed consumption when added to the broiler chicken diet at a concentration below 10g/kg of feed. Cholesterol levels in this study were not affected by the inclusion of the probiotic and the enzyme. In contrast, Tengfei et al. (2021) demonstrated a decrease in cholesterol levels with the addition of yeast.

They hypothesized that live yeast inhibits the oxidation of cholesterol, leading to reduced lipid deposition in blood vessels. Consequently, live yeast may exert an anti-cholesteremic effect in broilers. However, the mechanism by which dietary yeast cell inclusion lowers cholesterol in broilers is still under investigation.

The animals fed with *S. boulardii* RC009 plus phytase exhibited the highest values of phosphorus digestibility, followed by those fed with phytase alone. These results are in agreement with those of different authors who reported that the addition of probiotics to diets contribute to improve the digestibility and the uptake of nutrients (Anggraeni et al. 2019; Biswas et al. 2023).

For evaluate the answer of probiotics on intestinal morphology and cell proliferation are usually used histomorphometric parameters, such as the length of the villi, the depth of the crypt, the villus/crypts ratio and the surface area of the villi, been considered indicators of intestinal functions. In this work, the height and width of the intestinal villi, crypt depth and apparent absorption area were positively influenced by the probiotic yeast plus phytase. These results agree partially with those reported by different authors who demonstrated the beneficial effect of the use of probiotics in chickens on villus height, crypt depth, higher villus height-crypt depth ratio, etc., which indicates an increase in nutrient absorption by increasing the available surface area for nutrient absorption (Jha et al. 2020; Poloni et al. 2020). Prior studies have indicated that incorporating the probiotic S. *cerevisiae* into pig diets demonstrated a tendency to improve histomorphometric parameters in the intestine (Poloni et al. 2020). Similar findings were observed in a study by Jha et al. (2020), indicating that supplementing the probiotic S. cerevisiae had a favorable impact on histomorphological measurements of small intestinal villi in broiler chickens. This supplementation led to an increase in villus height and the villus height to crypt depth ratio.

The feed additives must demonstrate that it does not exert genotoxicity., A successful method in the assessment of chromosome damage, genotoxicity and cytotoxicity is the micronuclei assay (Mañas et al. 2009; Sabini et al. 2013). The bone marrow micronuclei assay in broiler chickens was conducted to assess the safety and potential genotoxicity of the probiotics and/or prebiotics intended to be used as additives in animal feed. It is important to state that *S. boulardii* RC009 and phytase did not cause any increase in the number of micronucleus erythrocytes.

On the other hand, the results obtained in this study agree with the findings of Magnoli et al. (2021) showing that the inclusion of 1g/kg of *P. kudriavzevii* RC001 in broiler chicken diets were neither cytotoxic nor genotoxic.

5. CONCLUSION

The utilization of *S. boulardii* RC009 alone or in combination with phytase resulted in significant improvements in productive parameters, carcass weight of economically important cuts and histomorphometric parameters in the small intestine. Furthermore, these additives did not exhibit toxicity. These results indicate their promising potential for standalone use or in combination and suggest they could serve as alternatives to antibiotic growth promoters.

Conflict of 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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