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ARTÍCULO DE INVESTIGACIÓN

Impact of *Saccharomyces boulardii* RC009 administration on clinical and microbiological parameters in Thoroughbred racehorses: a controlled study

Impacto de la administración de *Saccharomyces boulardii* RC009 sobre parámetros clínicos y microbiológicos en caballos de carreras pura sangre: un estudio controlado

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ABSTRACT

This study evaluated the effect of *Saccharomyces cerevisiae* var. *boulardii* RC009 on body condition, coat quality, fecal score, and fecal microbiota profile in 25 Thoroughbred racehorses over 30 days. Animals were divided into a control group (CG) and a treated group (TG), the latter supplemented with $2x10^{10}$ CFU/day. In the TG, coat condition improved significantly: 53.8 % increased by one grade, and the remaining 46.2 % by two grades. Regarding faecal consistency, 58.3 % of the CG and 53.8% of the TG increased their score by one point. Microbiologically, TG showed a relative increase in the families *Lactobacillaceae* and *Prevotellaceae*, taxa associated with optimized digestion. The results indicate that *S. boulardii* RC009 supplementation positively impacted body condition, coat appearance, and faecal microbiota profile. These findings suggest that the probiotic favorably influences host physiology and microbial composition, supporting the need for further longitudinal studies with larger cohorts to further explore these mechanisms.

Keywords: (productive parameters), (microbiome studies), (yeast), (horse)

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RESUMEN

Este estudio evaluó el efecto de *Saccharomyces boulardii* RC009 en la condición corporal, pelaje, puntuación fecal y el perfil de la microbiota fecal en 25 caballos pura sangre durante 30 días. Los animales se dividieron en un grupo control (GC) y un grupo tratado (GT), este último suplementado con $2x10^{10}$ UFC/día del probiótico. En el GT, el pelaje mejoró significativamente: el 53,8 % aumentó un grado y el 46,2% restante, dos grados. Respecto a la consistencia fecal, el 58,3 % del GC y el 53,8% del GT aumentaron su puntuación en un punto. Microbiológicamente, el GT mostró un incremento relativo en las familias *Lactobacillaceae* y *Prevotellaceae*, taxones asociados a una optimización de la digestión. Los resultados indican que la suplementación con *S. boulardii* RC009 impactó positivamente en la condición corporal, el aspecto del pelaje y el perfil de la microbiota fecal. Estos hallazgos sugieren que el probiótico influye favorablemente en la fisiología del huésped y en la composición microbiana, respaldando la necesidad de estudios longitudinales adicionales con cohortes mayores para profundizar en los mecanismos.

Palabras clave: (parámetros productivos), (estudios del microbioma), (levadura), (equino)

INTRODUCTION

Optimizing dietary composition is a key factor in enhancing animal health, welfare[1], productivity, and livestock performance[19,14]. This is important, especially in sports animals, such as Thoroughbred racehorses (TB).

High-performance horses are frequently subjected to intensive management practices, including high-starch diets, limited grazing, and psychological stress related to training and transport. These factors significantly predispose animals to hindgut dysbiosis, which can lead to metabolic disorders, systemic inflammation, and a subsequent decline in athletic performance.

In this context, the equine industry represents a strategic sector for Argentina, one of the leading global producers of Thoroughbred horses. The country consistently ranks among the top foal producers worldwide, driven by a robust market and international racing competitiveness. Given the high genetic and economic value of these animals, implementing advanced nutritional strategies, such as the use of specific probiotics, is essential to maintain the country's prestige and economic stability within the global equestrian market[25]. Consequently, maintaining stable and resilient intestinal microbiota is essential for both physiological health and competitive efficiency. The use of probiotics as feed additives has demonstrated positive effects on animal productivity and health, such as modulation of the immune system, stimulation of digestive enzyme activity, stabilization of the intestinal mucosal barrier, prevention of colonization by microbial pathogens, and adsorption of mycotoxins in the intestine[2].

The primary target sites for probiotics in horses are typically the cecum and/or colon. To date, many equine probiotics are marketed based on studies conducted in humans; however, given the significant differences in gastrointestinal physiology and anatomy, specific studies are necessary. Previous studies have demonstrated the probiotic properties of *S. boulardii* RC009 in other species[20, 17]. Unlike bacterial probiotics, *Saccharomyces* species—specifically *S. boulardii* —have the unique ability to compete for binding sites with enteric pathogens and serve as oxygen scavengers in the hindgut. This creates a more favorable anaerobic environment for cellulolytic bacteria, potentially enhancing the fermentation of structural carbohydrates[6,7]. Despite these promising attributes, scientific evidence regarding the use of specific autochthonous or well-characterized strains like RC009 in Thoroughbred horses remains scarce, necessitating targeted research to validate its efficacy in this specific physiological context. Therefore, we hypothesize that the consumption of additives based on this probiotic may improve production and health indicators in horses.

The present study aimed to evaluate the differences in body condition score (BCS), coat condition, faecal score, as well as faecal microbiota profiles, between probiotic-supplemented (Saccharomyces boulardii RC009) and control Thoroughbred athletic horses.

MATERIALS AND METHODS

Ethical approval

The working protocol and the techniques used comply with the regulations of the Animal Bioethics Subcommittee under the Scientific Research Ethics Committee of the National University of Río Cuarto (Approval Nº 383/22).

Probiotic microorganism and yeast biomass production

The probiotic *Saccharomyces cerevisiae var. boulardii* RC009 was isolated from the intestine of a pig. Its probiotics and molecular properties were analysed as described by Armando *et al.*[2]. The species identification was carried out using the Yeast Identification Database (https://www.yeast-id.com). Sequence comparisons were performed using the Basic Local Alignment Search Tool (BLAST) in the NCBI database, and the resulting data were submitted to GenBank (accession number KF447149.1). The propagation of the yeast was carried out in a fermenter (New Brunswick Scientific Co., Inc., En^{2}eld, CT, USA) for biomass production, following the methodology proposed by Poloni *et al.*[20].

Animal housing

This study was conducted at a stable located in the Mendoza Racetrack (Argentina), from December 2022 to January 2023, during the break in the competitive season. The fieldwork lasted a total of 30 days, coinciding with the warmest months of the year, when average maximum temperatures reached 33 °C and minimum temperatures dropped to 19 °C. The stable features 27 enclosed pens (3.50 x 3 m), each with a roof, concrete floors covered by a 20 cm layer of wood shavings, individual waterers, and elevated concrete feeders. Additionally, there was a functional chute and a feed storage area. All animals had a minimum daily training to maintain their sporting status.

Twenty-five (25) apparently healthy Thoroughbred (TB) horses of medium and high performance (6 females and 19 males), aged between 3 and 7 years, with an average body weight (BW) of 484.72 ± 37.62 kg, were selected. All the horses involved in the study were dewormed with Ivermectin-Praziquantel (Equiverm of Richmond VetPharma TM) according to the manufacturer's recommendation (without prior coprological examination); they had a negative Coggins test for equine infectious anemia within the past 60 days, and they were vaccinated against the equine influenza virus.

Experimental design and evaluated parameters

The animals were randomly assigned to two groups: a control group (CG) of twelve (12) horses that were not supplemented with probiotics, and a treatment group (TG) of thirteen (13) horses that were orally administered with 1 g of *S. boulardii* RC009 at 2 x 10^{10} CFU/day, directly into the horse's mouth for 30 days. The feeding regimen for each horse was individualized based on their body weight (2 % BW). The diet consisted of a base diet (BD) of alfalfa forage (PB 18%, EM 2.40 Mcal/kg, FDA 32%, FDN 40 %, MS 86 %) and soaked oat grain (PB 11.5 %, EM 2.48 Mcal/kg, MS 91%), with an alfalfa/oat ratio of 55/45. Alfalfa forage was provided at 12:00 am and 7:00 pm, followed by grain oats soaked in a 2:1 water ratio for 2 hours. Water was changed three times daily (morning, afternoon, and night) to ensure it remained clean, fresh, and readily available.

The measurement of body condition score (BCS) was conducted weekly using the body condition scoring system according to Henneke *et al.*[5]. This system assesses fat cover on a scale ranging from 1 to 9, using both visual evaluation and palpation. A body condition score between 5 and 6 was considered ideal for healthy horses. The faecal score of each horse was evaluated in situ at the time of defecation weekly. They were graded on a scale from 1 to 5 according to Laustsen *et al* [8]. The same observer inspected the coat condition weekly (W0-W3) throughout January, following the completion of the winter coat shedding. Coat condition (CS) was graded on a scale from 1 to 3 according to Mthi *et al.*[15].

At the end of the experimental period, feces samples from horses in each group were taken by anal stimulation, pooled by treatment (two pools/treatment), and transported immediately and frozen at -80 °C until analysis by metagenomics. Total DNA was extracted and purified using the QIAamp DNA Stool Mini Kit (Qiagen), and microbial populations were analyzed by Next Generation Sequencing (NGS), performed by Novogen sequencing service, Sacramento, CA, USA, with the Illumina NovaSeq PE250 sequencing platform, using primers for amplification of the region 16S V34. Quality

filtering on the raw merged reads was performed using the fastp software to obtain high-quality sequences[3]. The final reads were then subjected to taxonomic profiling using the k-mer based tool Kraken2[28] and relative abundance estimation using Bracken[10]. Taxonomic classification and abundance estimation were performed using the SILVA database (SSU Ref NR 138.2) for Kraken2. Operative Taxonomic Units (OTUs) quantification and taxonomic analysis were used to assess α and β diversities.

Statistical analysis

Bivariate analyses to assess significant differences between health indicators according to treatment were evaluated using Fisher's exact tests and analysis of variance (ANOVA) for categorical and continuous variables, respectively. Means were compared according to Fisher's minimum significant difference test (LSD) (p<0.05)[21]. The analysis was carried out using the InfoStat program.

RESULTS

Body condition score, coat condition, and faecal score

Table 1 shows the BCS obtained weekly via inspection and palpation of fat-coverage areas in the control and treated groups. The animals in the CG maintained a BCS between 5.5 and 5.8 from the beginning to the end of the study. The animals in the TG showed significant differences in BCS during the tested weeks (p<0.05). Animals started with a BCS0 of 4.6 and ended with a BCS4 of 5.7. Regarding coat condition (CS), 33.3 % of the horses in the CG maintained their initial score, 58.3 % increased by one grade, and 8.3% showed a decrease. In contrast, 100 % of the horses in the TG showed a significant improvement in their CS: 53.8 % increased by one grade, while the remaining 46.2 % increased by two grades (Table 1).

Faecal score in CG had consistency between F2 - F4 at day 0 of the study and was between F3 - F4 at 15 days until the end of the study (Table 2). Whereas in TG the consistency was more variable; the trial started with values among F1 - F3, on day 15 they varied among F2 - F4, and ended with percentages among F1 - F4. In CG, 58.3 % of animals increased faecal consistency by one point. In the TG, 53.8 % increased the faecal consistency.

Table 1. Body condition scores are obtained weekly through inspection and palpation of fat coverage areas. Variations (%) of initial and final body conditions for control and treated groups.

	Weeks	CG	TG
		Scores	
Body condition score*	BC0	5.5 ± 0.7[a]	4.6 ± 0.7[a]
	BC1	5.6 ± 0.7[a]	5.0 ± 0.6[ab]
	BC2	5.6 ± 0.5[a]	5.2 ± 0.5[bc]
	BC3	5.7 ± 0.5[a]	5.4 ± 0.7[bc]
	BC4	5.8 ± 0.7[a]	5.7 ± 0.4[c]
Variations in body condition score	Animals	Percentage (%)	
Initial	1	0	0
	2	0	0
	3	0	7.7
	4	0	23.1
	5	58.3	69.2
	6	33.3	0
	7	8.3	0
	8	0	0
	9	0	0
Final	1	0	0
	2	0	0
	3	0	0
	4	0	0
	5	33.3	38.5
	6	50	53.8
	7	16.6	7.7
	8	0	0
	9	0	0

*CG-control group, TG-Treatment Group. BC0 corresponds to the initial BCS, and BC1, BC2, BC3, and BC4 to weeks 1, 2, 3, and 4, respectively. Common letters are not significantly different (p>0.05), and they must be read vertically.

Table 2. Faecal scores and variations of increase or decrease per group.

Groups	Days			Increase and decrease (%)	
	Faecal score				
	0 (Initial)	15 days	30 days	1P	2 P
CG	F3 (F2 - F4)	F3.5 (F3 - F4)	F3.5 (F3 - F4)	+ 58.3	
TG	F2 (F1 - F3)	F3 (F2 - F4)	F2.5 (F1 - F4)	+ 53.8	- 7.7

CG-control group, TG-Treatment Group. Scale from F1 to F5. F1 indicated very loose stools, and F5 indicated very dry and hard faeces.

Metagenomic

The Venn diagram shows the number of shared and different OTUs found in the CG (CG 1 and CG 2) and TG (TG 3 and TG 4), where the number of individual OTUs per sample was 39 - 60 and 48 - 66, respectively (Fig. 1).

The taxonomic composition was calculated using metagenomics methods at the phylum, family, and genus levels. In the equine faecal samples of both groups the most abundant bacterial phylum was *Firmicutes*, followed by *Verrucomicrobiota* and *Bacteroidota* in the CG, and in the TG by *Proteobacteria* and *Bacteroidota* (Fig. 2). At the family level, the CG was characterized by the presence of *Lachnospiraceae*, *Oscillospiraceae* and *Rikenellaceae*, while in the TG the predominant abundance of the *Lactobacillaceae* family was observed, followed by *Burkholderiaceae* (Fig. 2). Among the less abundant families, a higher relative abundance of *Campylobacteraceae*, *Clostridiaceae* and *Enterobacteriaceae* was observed in the CG compared to the TG, while the abundance of *Enterococcaceae* was higher in the faeces of the TG (Fig. 2). The linear discriminant analysis effect size (LEfSe) identified a higher relative concentration of *Lactobacillus*, *Ligilactobacillus*, *Ralstonia* and *Limosilactobacillus* in the TG compared to CG. While in the animals of the CG, a significantly higher presence of *Rikenellaceae* RC9 gut group, *Akkermansia, Eubacterium coprostanoligenes* group, *Bacteroides,* and *Christensenellaceae* R-7 group was observed.

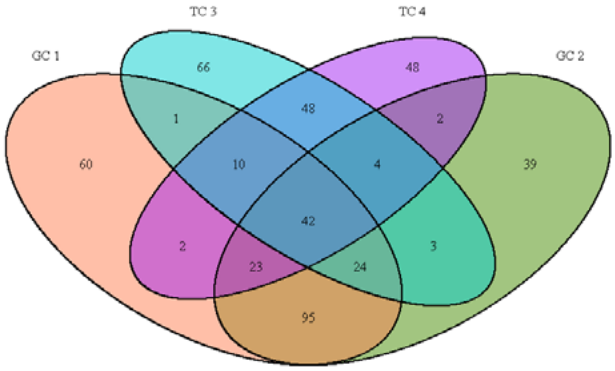

Figure 1. A Venn diagram shows the number of shared and different OTUs found in the Control Group (CG 1 and CG 2) and Treatment Group (TG 3 and TG 4). Each circle in the figure represents a sample, and the number in the circle represents the overlap between representative samples. The number of OTUs where no overlap represents the number of unique OTUs in the sample.

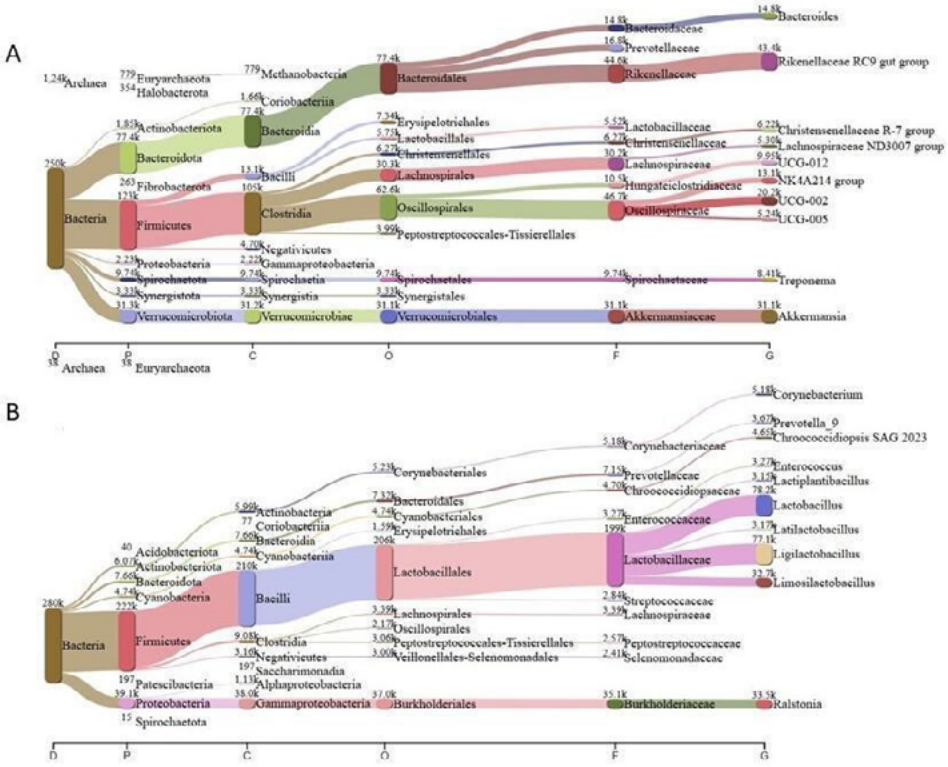

Figure 2. Diagrams showing taxonomic composition at all levels. Each level contains the top 10 most abundant taxa. Bar height is a proportional number of reads assigned to each feature after Bracken estimation. Readings for numbers are also shown on top of the bars. A) Control group, B) Treatment group.

DISCUSSION

For a long time now, several effects have been reported in equines with the use of yeast-based feed additives, such as the regulation of the normal digestion and fermentation process in the cecum, reduction of pH, and lactic acid increase after feeding starch-rich diets, or the regulation of the degree of undesirable changes in the intestinal ecosystem when the small intestine is saturated with starch[12]. They have also been associated with improved digestibility of the cellulose fraction, vitamin supply, especially B-complex vitamins, in addition to improved mucosal and systemic immunity[6, 4, 13], reduced incidence and duration of diarrhea[24], and mitigation of exercise-induced stress[26].

Body condition scoring systems were originally developed to quantify flesh cover in food animals and are commonly used to evaluate body fat in *Equidae*. It is a semiquantitative method of estimating subcutaneous fat coverage in many species and, particularly in the equine, is used as an index of energetic balance. An extremely high or low BCS can negatively affect athletic performance, with optimal ranges of 4 to 7 reported for different equestrian sports[16]. In the present study, 84.5 % of the supplemented horses increased adiposity in the explored areas, positioning themselves in the final BCS. No reports have been found in the literature on body condition measurements after probiotics use.

One of the most noticeable changes after the administration of the probiotic was the condition of the hair coat, which, in practice, is recognized as a readily observed indicator of the overall health of the individual. All the probiotic-supplemented horses improved their coat condition, and 92.5 % of them ended up with the maximum category. Different studies in the literature relate the condition of the hair coat to the use of feed additives in equines. Richards *et al.*[22] reported an improvement in hair coat quality in equines consuming feed additives with different oils. The profound effect of nutrition on hair coat quality is explained by the hair follicles, which are metabolically active tissues that require nutrients to support both structural and functional activities. Recently, in a study of cows, the health of the hair coat was associated with the composition of the gut microbiota and its interaction with host metabolism[29]. On the other hand, the results show that the administration of *S. boulardii* RC009 contributed to maintaining a

stable faecal consistency, demonstrating physiological stability in the stools, without any episodes of diarrhea or adverse clinical changes being recorded during the experimental period. Specifically, 53.8 % of the animals in the treated group showed an increase in faecal consistency score. This suggests that the probiotic helps stabilize the intestinal ecosystem, preventing the fluctuations in hindgut fermentation that often lead to digestive disorders in high-performance horses.

Metagenomic analysis of faeces showed different bacterial population profiles among the animals that consumed the additive. Although *Firmicutes* and *Bacteroidetes* were the first and third most abundant phylum in both groups, the second most abundant phylum *(Proteobacteria)* in faeces was different in the treated group and the control group. Coinciding with previous studies, *Verrucomicrobiota* was the second most abundant in the control group, with a high relative abundance of *Akkermansia*, which has been proposed to be of importance for its anti-inflammatory properties demonstrated in humans[30]. In the faeces of treated equines, the second most abundant phylum was *Proteobacteria*. Microbial diversity was higher in the horses with our probiotic yeast. Similar results were reported by Lucassen *et al.*[11], in a study that compared the alpha diversity of the faecal microbiota of equines consuming an additive based on *S. cerevisiae*, in comparison with a group of equines consuming a placebo, after the animals were vaccinated against influenza.

The main relative growth in the probiotic-treated animals was in the Lactobacillaceae family. *Lactobacillus* is one of the most studied microorganisms for its probiotic effects. Different studies have reported the effect of various species in improving the functionality of the intestinal barrier and maintaining intestinal homeostasis[27]. Different *Ligilactobacillus* species have been associated with beneficial effects, such as antibacterial activity or regulation of intestinal biota. However, its main role has been associated with the enzymatic degradation of cellulose[9]. This makes it a very important population for the complete utilization of the consumed feed. In the present work, the use of probiotic *S. boulardii* increased the population of *Ligilactobacillus* in the faeces, which is therefore an interesting finding that may be related to the increase in body condition in animals receiving the probiotic yeast. In agreement with previous studies, the use of an additive with S. *boulardii* RC009 also increased the relative abundance of *Prevotellaceae*, another microorganism with high cellulolytic activity[17]. *Lachnospiraceae* and *Prevotella* have been reported to be more abundant in the faeces of high-performance sport horses compared to low-performance ones[18]. In the present work, the relative abundance of *Lachnospiraceae* was reduced in probiotic-treated animals. In contrast to what was proposed by Schoster (2018)[23], this reduction seems not to have been associated with gastrointestinal disorders, as no clinical signs or changes in stool consistency were evident.

On the other hand, in the control group, *Eubacterium coprostanoligenes group*, *Christensenellaceae* R-7 group, and *Rikenellaceae* RC9 gut group were the populations of beneficial bacteria present, but there were also found a higher number of other bacteria associated with gastrointestinal disorders in equines, such as *Bacteroidetes* in agreement with Kauter *et al.*[7].

Despite the positive outcomes, some limitations should be acknowledged. The assessment of BCS and coat quality remains inherently subjective. Furthermore, the absence of baseline (Day 0) faecal microbiota sampling prevents the establishment of individual microbial trajectories. Since faecal samples serve only as a proxy for the complex gastrointestinal ecosystem, these results should be interpreted as preliminary evidence.

CONCLUSION

In conclusion, supplementation with *Saccharomyces boulardii* RC009 effectively improved coat condition and body score in athletic Thoroughbred horses while modulating the fecal microbiota. The observed enrichment of *Lactobacillaceae* and *Prevotellaceae* suggests an optimization of fiber-degrading populations and overall gut health. These findings support the use of *S. boulardii* RC009 as a functional probiotic tool to enhance the physiological status and intestinal balance of horses under high-performance conditions.

CONFLICTS 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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AUTHOR CONTRIBUTIONS

Ricardo Ludueña: resources and methodology; Danilo Ceschin: software and formal analysis; Lilia Renée Cavaglieri: Project administration, resources, funding acquisition and conceptualization.
Alejandra Paola Magnoli: writing – original draft and visualization; Laureano Jose Giordano: software and formal analysis; Ana Laura Dávila: investigation; María Julieta Luna: investigation;

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