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Use of the Probiotic *Saccharomyces Cerevisiae* Var. *Boulardii* RC009 in the Rearing Stage of Calves

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ABSTRACT

The objective of this study was to determine the influence of the application of the probiotic *Saccharomyces cerevisiae var. Boulardii* -RC009 to different times on productive parameters of calves in the feedlot in the rearing stage. The concentration of *S. boulardii* RC-009 in the probiotic additive was $1x10^{10}$ CFU/g, mixed with the diet to obtain a dose of 1 g/animal/day. Treatments: T1- control (TMR without probiotic additive); T2- Probiotic (TMR with $5x10^{11}$ CFU/t *S. boulardii* -RC009). Experiment 1: 82 weaned calves (Aberdeen Angus) of 9-10 months old (males and females) with an initial weight of 175 and 183.5kg were used. The animals were weighed and separated into two groups of 41 animals each. The animals were weighed on days 1, 34, and 53 days of the experiment. Experiment 2: 140 Aberdeen Angus calves 12 months old (males and females) with an initial weight of 260-291kg were used. The animals were weighed and separated into 2 groups of 70 animals each. The animals were weighed on days 1, 18, and 35 days of the experiment. Calves from experiment 1, showed a conversion efficiency of 4% compared to the control animals (7.1-7.4: 1, respectively). Calves from experiment 2, showed a conversion efficiency of 3.77% compared to control animals (5.1-5.3:1, respectively). In conclusion, this is the first time that a product based on *S. boulardii* -RC009 demonstrates a better conversion efficiency in crossbred feed-lot calves in the rearing stage with an administration period of 35 days, positively impacting production costs.

Key words: Probiotic, *S. Boulardii* -RC009, Weaned Calves, Productive Performance.

INTRODUCTION

The importance of carrying out improvements in cattle production is becoming more and more necessary, due to the fact that the demand for by-products obtained from these is increasing with the passing of days, it is important to note that a variety of strategies have been used to carry out said action, within which is the use of antibiotics as growth promoters, these in turn have been prohibited due to the consequences it generates in the health of consumers (Chaucheyras-Durand and Durand 2010). There is a possibility to replace the use of antibiotics as growth promoters with the following groups of feed additives: a) technological (binders and preservatives), b) coccidiostats, c) Sensory (coloring and flavoring), d) nutritional (vitamins and amino acids) (Caja et al. 2003; Rashid et al. 2023).

The prohibition and limitation of the use of antibiotics as growth promoters in world production have prompted research into the inclusion of microbial additives for animal nutrition. In this sense, the probiotics within the group of zootechnical additives have been extensively studied, being one of the most widely used alternatives to replace antibiotics, providing additional benefits for the animal production system (Adjei-Fremah et al. 2015; Serwecińska

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2020; Gul and Alsayeqh 2023). Several researchers have reported the benefits of oral administration of probiotics in ruminants. Probiotics regulate and balance the intestinal biota, promote the growth and development of animals, and improve resistance to disease (Xu et al. 2017; Mandouh et al. 2020). Recent studies suggest that the use of probiotics as a supplement in feed for ruminants improves growth performance, and production and improves the health and general welfare of animals (Adjei-Fremah et al. 2015; Coniglio et al. 2023). Feeding calves probiotics at weaning can facilitate the development of bacterial communities in the rumen and help calves transition from liquid to dry feed and forage; In addition, *Saccharomyces cerevisiae* fermentation products have demonstrated a positive influence on the rumen microbiota and the improvement of rumen morphology (FAO/WHO 2001). Probiotics in adult ruminants improved fiber digestion by ruminal microorganisms, positively affecting productive parameters, and improving digestive processes (especially cellulolysis), and microbial protein synthesis (Uyeno et al. 2015; Gul and Alsayeqh 2022; Gul and Alsayeqh 2023). After an extensive review of the literature, little data is available on the use of *Saccharomyces cerevisiae* var. *boulardii* in calves after weaning.

Therefore, the objective of this study was to determine the influence of the in-feed inclusion of probiotic *Saccharomyces cerevisiae* var. *boulardii* -RC009 at different times on the productive parameters of crossbred calves in a feedlot in the rearing stage.

MATERIALS AND METHODS

Ethical Approval

The experience was subject to approval by the Ethics Committee of the National University of Rio Cuarto, and compliance with the regulations of the Subcommittee on Animal Bioethics under the Ethics Committee of Scientific Research.

Probiotic Additive Formulation

Saccharomyces cerevisiae var. boulardii RC009 (*S. boulardii*) was isolated from the intestine of a healthy pig (Armando et al. 2011). This strain was deposited in the Collection of Industrial Microbiology, Biotechnology Applied to the production of Animal feed additives group (BIOAPLA) from the National University of Río Cuarto.

Saccharomyces boulardii biomass was obtained from 24h culture in Yeast-Peptone-Dextrose (YPD) broth in a BioFlo 2000 fermenter (New Brunswick Scientific Co., Inc, Enfield, CT, USA) operated at 28ºC, at 1x*g* and 1.5vvm aeration. The pH value was adjusted to 5 with 6M NaOH. The working volume was 4L. The biomass obtained at the end of the fermentation process was centrifuged at 5000×*g* at 4°C for 10min. The concentrated pellet was re-suspended in the same volume of cryoprotectant (2.5% maltodextrin), frozen at –80°C, and lyophilized. Lyophilized biomass (1g) was hydrated, and the viability was confirmed by counting the colony-forming units (CFU/g) counts. Lyophilized *S. boulardii* biomass was considered the probiotic additive. The concentration of *S. boulardii* RC009 in the probiotic additive was $1x10^{10}$ CFU/g. The probiotic additive (50g) was mixed with the corresponding diet per ton ($5x10^{11}$ CFU/t).

Experimental Design

The experiments were carried out in a feedlot located in Río Cuarto Department, Córdoba Province, MAREUBA S.A with the following geographical location (latitude -33.323037; length - 64.419173). The animals (N=222) were grown in pens 50m wide and 70m long, with feeders on concrete stands 3m long, and a steel cable that prevented the animals from entering the feeder. The floor was well compacted, with rest areas, and a slope to settle solids and liquids. Each pen had a unique drinking point that provided enough clean and fresh water.

Two experiments were realized to determine the influence of the in-feed inclusion of the probiotic *S. var. Boulardii*-RC009 at different times on productive parameters of crossbred calves in a feedlot in the rearing stage. The feed used for both experiments consisted of a Totally Mixed Ration (TMR), whose composition was as follows: corn silo 32%; cracked corn grain 41%; Wet Distillers Grains (WDG) 25%; corrector 2%; dry matter of TMR 58%. Diets were formulated to meet the nutrient requirements according to the NRC (2001).

The calves (N=222) were fed with 4.16kg of DM (dry matter) with or without probiotic additive per animal, twice a day (9:00 am and 5:00 pm). An electronic chip was placed in the ears of the animals to monitor them using a commercial system called Tru-test.

The dietary treatments in both experiments used were the following: T1- control (TMR without probiotic additive); T2- Probiotic (TMR with $5x10^{11}$ CFU/t *S. boulardii* -RC009).

Experiment 1: Eighty-two (82) crossbred calves (Aberdeen Angus – Hereford F1 Cross) 9-10 months old (male and female) with an average initial weight of 175.6±11.59, and 183.5±38.3kg were used. Animals were weighed and divided randomly into two equal groups. The dietary treatments (T1-T2) were used for 63 days. The productive parameters were evaluated on days 1, 34, and 63 of the experiment.

Experiment 2: One hundred forty (140) crossbred calves (Aberdeen Angus – Hereford F1 Cross) 12 months-old (male and female) with an average initial weight of 260.1±31.0, and 291.4±27.3kg were used. Animals were weighed and randomly divided into equal two groups. The dietary treatments (T1-T2) were used for 35 days. The productive parameters were evaluated on days 1, 18, and 35 of the experiment.

Health Plan Applied in Each Experiment

The health plan in each experiment was according to the feed-lot protocol. Double dose vaccine against clostridia and pneumonia, with an interval of 15 days; Single dose of copper and zinc on admission; Parasites one dose on admission ivermectin; Argentine national plan for the control and eradication of Aphthous disease.

Productive Parameters Determination

Daily dry matter intake (DDMI) was estimated per pen from the daily supply of feed. Then, the group's daily intake was divided by the number of animals present in the pen. Weight gain (WG) was calculated as the difference between the final weight and the initial weight of each animal. Daily weight gain (DWG) was calculated as the weight gain of each animal (WG) divided by the period in days; it was estimated individually**.** Conversion index (CI) was calculated by dividing the amount of DDMI (kg) by the DWG.

Statistical Analysis

The productive parameters WG, daily DWG, and CI were determined using each animal as an experimental unit. Data were analyzed by the general linear and mixed model (GLMM) using Statistical Analysis System software (InfoStat 2012, Cordoba University, Argentina). Means and standard deviation were compared using the Fisher's protected least significant test (LSD) ($P<0.001$).

RESULTS

The results the means of productive parameters of experiment 1 and 2 of crossbred calves fed with and without *S. boulardii*-RC009 during the experimental period of 35 and 63 days were shown in Table 1, Fig. 1, and 2. The values of productive parameters not showing significant differences between the control and probiotic treatments in both experimental periods ($P\geq0.001$); however, it is important to highlight that the daily weight gain values showed a positive trend with the use of *S. boulardii* RC009 during the experimental period of 35 days. Also, crossbred calves of 12-month-old showed a conversion efficiency of 4% compared to control animals (7.1-7.4: 1 respectively), this means that each animal needs 300g less of DM to produce one kg of meat.

The cost of TMR in the dry base of this study was 100USD/t. The cost of including probiotics in diets in Argentina is approximately 85USD/kg, in this study, 50g of the probiotics were added per T of food, which represents a cost of 0.023USD/kg, that is, the total cost of the TMR was 94USD/t.

The 300g less food consumed by the 41 animals in the 35 days of the experiment represents a total of 430kg of TMR DM with a cost of 94USD. This means that in this trial the cost of the total TMR used was 41 dollars less, compared to animals that did not consume the probiotics. On the other hand, calves from experiment 2 also showed a 3.77% conversion efficiency lower compared to control animals (5.1-5.3:1, respectively).

DISCUSSION

The objective of this study was to determine the influence of the inclusion of probiotic *S. cerevisiae* var. *boulardii* RC009 at different times on the productive parameters of crossbred calves at different rearing stages.

Table 1: Productive parameters (kg) of crossbred calves (Aberdeen Angus – Hereford F1 Cross) of different ages (9-10 and 12 months-old) fed with and without *S. boulardii*- RC009 during the experimental period of 35 and 63 days.

Age/Productive Parameters	Day of Assays	With Probiotics	Without Probiotics
9-10 Months Old			
W	1 (initial)	175.6±11.6a	183.5±38.3a
	34	273.1±37.9a	271.9±31.1a
DWG	34	1.52±0.3a	1.50±0.5a
DWG	63	1.53±0.3a	1.53±0.2a
CI	63	5.1:1	5.3:1
12 Months Old			
W	1 (initial)	291.4±27.3a	260.1±31.0a
	18	323.8±28.8b	288.0±30.5a
DWG	18	1.8±0.3a	1.5±0.2a
DWG	35	1.7±0.4a	1.6±2.1a
CI	35	7.1:1	7.4:1

Values (mean±SD) bearing different letters in a row indicate significant differences ($P\leq0.001$). W: Weight; DWG: Daily weight gain; CI: Conversion index.

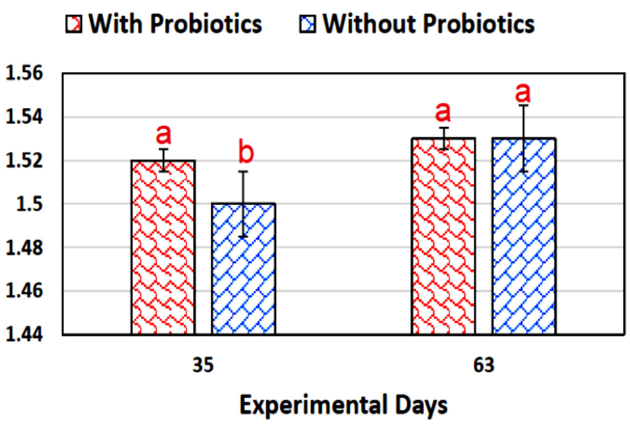

Fig. 1: Effect of *S. boulardii*- RC009 supplementation on daily weight gain (kg) during the experimental period of 35 and 63 days of 9-10 months-old calves. At day 35, daily weight gain was significantly ($P<0.05$) higher in probiotics group than without probiotics.

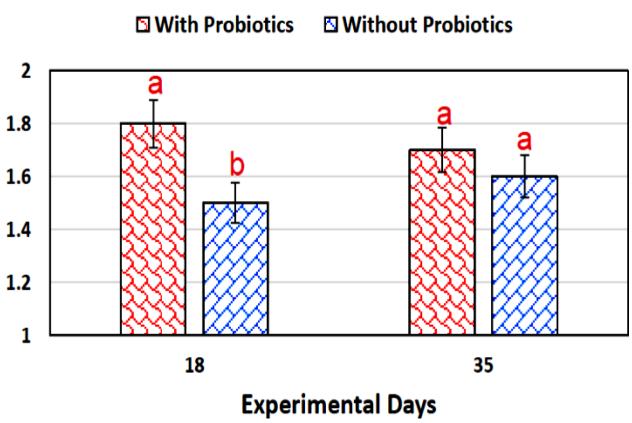

Fig. 2: Effect of *S. boulardii*- RC009 supplementation on daily weight gain (kg) during the experimental period of 18 and 35 days of 12 months-old calves. At day 18, daily weight gain was significantly ($P<0.05$) higher in probiotics group than without probiotics.

Probiotics are currently the most widely used alternative, due to the number of restrictions and repercussions that the use of antibiotics as growth promoters has been having, which has generated the implementation of new diets and feeding systems in large bovine productions (Sánchez Miranda and Peña Rodríguez 2016).

This study showed a positive trend in the DWG in 12-month-old calves that received *S. boulardii* -RC009 during the 35-day experimental period. On the other hand, it was shown that the DWG was not modified by the administration of *S. boulardii* -RC009 during a period of 63 days to 9-month-old calves.

These results are partially in agreement with (Penha et al. 2011), who reported a higher WG in 18-month-old male cattle fed 4g of *Brachiaria brizanthay*/animal/day as probiotics and enzymes (amylase, cellulose, protease, lipase, pectins) for 150 days compared to cattle that did not receive probiotics. Kelsey and Colpoys (2018) demonstrated an improvement of the productive parameters in weaned calves fed with *Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei, and Lactobacillus plantarum* as probiotics (10 g/animal/day) for 3 weeks. Similarly, Adams et al. (2008) showed an increase in weight gain in Holstein-Friesian male calves fed for 2 weeks with *Propionibacterium jensenii* 702 (1.1×10^8 and 1.2×10^9 CFU/kg/day) before and during weaning compared to those who did not receive the probiotic. On the other hand, Zhang et al. (2016) did not report improvements in average daily gain, dry matter intake, and nutritional digestibility in Holstein calves supplemented with *Lactobacillus plantarum* GF103 (1.7×10^{10} CFU/animal/day and *Bacillus subtilis* B27 (1.7×10^8 CFU/animal/day), but they did report an improvement in CI of calves fed with *L. plantarum.*

Considering the inclusion of yeast, our results agree with the findings of Lesmeister et al. (2004) who demonstrated that the inclusion of 2% of *S. cerevisiae* in the feed of 2-day-old calves for 42 days of the trial improved the DWG by 15.6%, compared to the treatment without probiotics. Also, in previous studies, Coniglio et al. (2023) demonstrated an improvement in WG whit the inclusion of 50g of *S. boulardii* RC009 in the feed of weaned calves of 7-8 months old during an experimental period of 35 days. In any animal production system, diet is the central component in the definition of costs. The results obtained on the conversion efficiency showed a beneficial effect on the cost of feeding with the addition of *S. boulardii* RC009. These results agree with Khan et al. (2022) who reported that the addition of yeast *S. cerevisiae* 2g/day/animal has a positive effect on the cost of feeding in Damani goat kids.

Therefore, it is important to consider different strategies for the maximum use of each nutrient in the feed by the animal. It is known that the mode of action of probiotics involves changes in rumen fermentation rates and patterns, being effective in raising and stabilizing rumen pH by stimulating certain populations of ciliated protozoa, which quickly consume starch and, therefore, compete effectively with lactate-producing bacteria (Uyeno et al. 2015; Mousaie 2021; Ismael et al. 2022). It was also shown that the presence of *S. cerevisiae* benefits the growth of cellulolytic microorganisms and therefore the degradation of the fiber (Dogi et al. 2011).

In addition, yeasts provide growth factors for rumen microorganisms, including organic acids and oligosaccharides, B vitamins, and amino acids, which stimulate microbial growth in the rumen, indirectly stabilizing rumen pH (Uyeno et al. 2015; Emu et al. 2021).

Conclusion

In conclusion, this is the first time that a product based on *S. boulardii* RC-009 demonstrates a better conversion efficiency in crossbreed feed-lot calves in the rearing stage with an administration period of 35 days, positively impacting production costs.

Conflicts of interests

The authors declare that the present study had no conflict of interest.

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