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THE IMPACT OF *SACCHAROMYCES CEREVISIAE VAR. BOULARDII* RC009 ON PRODUCTIVE PARAMETERS IN WEANED CALVES AND CULL COWS

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ABSTRACT

In ruminants, the probiotics stimulate rumen fermentation, fed digestibility, degradability, and rumen microbiota. This study aims to evaluate the adaptation and performance of post-weaning calves and cull cows by the inclusion of probiotic *Saccharomyces cerevisiae var. boulardii* RC009 to food. Lyophilized. *S. boulardii* (1 x 10^{10} CFU/g) (50g) was mixed with the diet to obtain 5 x 10^{11} CFU/T. Dietary treatments were, T1- control (without probiotic), T2 - Probiotic (with probiotic). Experiment 1: 50 six-month-old, weaned calves (Aberdeen Angus – Hereford F1 Cross). The assay started when they were 7-8 months old and, between 130-146kg initial weights. Animals were weighed and divided into 2 groups (25 animals each). The dietary treatments were used for 35 days from weaning. Experiment 2: 80 six-month-old cross-cull cows (Aberdeen Angus – Hereford) with an initial weight between 427-456kg. Animals were separated into 2 groups (40 animals each). Dietary treatments were applied for 22 days. The productive parameters were determined at 35 days in each animal as an experimental unit. These parameters from the experiments showed that weight gain (WG) and daily weight gain (DWG) were significantly better in animals that received the probiotic additive, showing around 5 times greater WG. Feed Conversion was lower in animals supplemented with the probiotic additive, showing best efficiency. Moreover, control cull cows had similar WG, regardless of the breed. However, animals treated with probiotics, mainly those from Aberdeen Angus and Hereford breeds, had significantly higher WG. This is the first time that an *S. boulardii* RC009-based product demonstrated to be effective in improving productive parameters in both calves subjected to wean stress and cull cows. A positive impact on the reduction of costs related to the feeding of animals should be obtained since an increase in DWG was correlated with an improvement in feed conversion rate (FCI)..

Keywords: Probiotic *Saccharomyces boulardii*, Weaned calves, Cull cows, Performance

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1. INTRODUCTION

The production of cattle is an important source of animal proteins worldwide, as they cover nutritional needs with forage, thereby occupying a privileged position by not directly competing with food intended for humans (Adjei-Fremah et al. 2015).

Europe banned the use of growth-promoting antibiotics (GPA) in animal feed due to the potential risk of bacterial resistance to antibiotics, including the contamination of by-products such as milk or meat with their residues (Hong et al. 2005; Serwecińska 2020). As a result, different strategies have been proposed to replace GPA. The primary strategy to modify ruminal fermentation is the combination of the food ingredients, their composition, and relative proportion in the diet. Alternatively, natural or biological options have been developed to optimize the production efficiency by modifying ruminal fermentation, and some have been evaluated to replace the GPA (Adjei-Fremah et al. 2015; Carro and Ungerfeld 2015; Retta 2016; Bąkowski and Kiczorowsk 2021). Probiotics are defined by the Food and Agriculture Organization of the United Nations and World Health Organization as “live microorganisms which,

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when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO 2001). In ruminants, lactic-acid bacteria strains such as *Lactobacillus, Bifidobacterium, Bacillus, Saccharomyces,* and *Enterococcus sp*. are commonly used as probiotics in feedstuffs (Retta 2016; Bąkowski and Kiczorowsk 2021; Khan et al. 2022). Bacteria are used in young animals (pre-ruminants up to six months of age) whereas yeasts are mainly used in animals with functional rumen (growing and fattening beef cattle and lactating cows) (Diao et al. 2019).

The use of probiotics in ruminants/birds promotes the growth and development of animals and improves the host resistance to diseases due to its mechanism of action on gut microbiota as well (Adjei-Fremah et al. 2015; Xu et al. 2017; Diao et al. 2019; Ismael et al. 2022; Gul and Alsayeqh 2023). Administering probiotics to calves at weaning can facilitate the development of rumen bacterial communities and helps calves with the transition from liquid feed to dry feed as forages. In adult ruminants, probiotics can not only improve fiber digestion by ruminal microorganisms, and digestive processes (especially cellulolysis) but also positively influence productive parameters such as microbial protein synthesis (Uyeno et al. 2015). However, there are limited studies available on the use of *Saccharomyces cerevisiae* var. *boulardii* in post-weaning calves and adult ruminants. Therefore, the present study aimed to evaluate the adaptation and performance of post-weaning calves and cull cows by the feed inclusion of probiotic *Saccharomyces cerevisiae var. boulardii* RC009.

The probiotic's influence on cull cattle performance and breed was also studied. It was hypothesized that cattle fed with *Saccharomyces cerevisiae* var. *boulardii* RC009 impacted positively adaptation and performance when compared to cattle that did not receive the probiotic.

2. MATERIALS AND METHODS

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.

2.1. Probiotic additive formulation

The probiotic *Saccharomyces cerevisiae var. boulardii* RC009 (*S. boulardii*) was previously isolated from the intestine of a healthy pig by Armando et al. (2011). This strain is 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.

Yeast biomass was obtained following the methodology proposed by Fochesato et al. (2018). The viability of the lyophilized biomass was evaluated by counting the colony-forming units (CFU/g). The concentration of *S. boulardii* RC009 was 1 x 10^{10} CFU/g. Fifty grams (50g) of the probiotic was mixed with the corresponding diet per ton (5 x 10^{11} CFU/T).

2.2. Cows, treatments, and experimental design

The experience was carried out at the "El Cuatro" farm located in Rio Cuarto Department, Córdoba Province. The animals were kept in pens 50m wide and 70m long, with feeders on 3-meter-long concrete stands, and a steel cable that prevented the animals from entering the feeder. The floor was firmly packed, with lying areas, and a slope to drain away urine and feces. Each pen had a unique drinking point that provided enough clean and fresh water.

Experiment 1: was conducted with fifty 6-month-old weaned calves (Aberdeen Angus – Hereford F1 Cross). Animals started the trial at 7-8 months old (males and females) and, had an initial weight between 130 and 146kg. They were weighed and divided into two groups, twenty-five animals each. The feed consisted of a textured calf starter and rearing a basal diet (BD). The dietary treatments were used for 35 days from weaning; and designed as T1 – control: the animals received the basal diet without probiotic additive and T2 – probiotic: the animals received the same diet with the addition of *S. boulardii* (5 x 10^{11} CFU/T).

Diets were formulated to meet the nutrient requirements according to the NRC (2001). During the first 7 days of post-weaning, calves were fed with 4.16kg dry material (DM) of textured calf starter (with or without probiotic additive) per animal, twice a day (9:00 a.m. and 5:00 p.m.). On the 8^{th} day of the assay, 1kg of corn grain, and alfalfa roll (*ad libitum*) were added to the diet. On the 18^{th} day of the assay and until the end of the experiment (35 trial days), the calves were fed with 4.16kg DM of textured calf rearing (with or without probiotic additive), plus 1kg DM of corn grain per animal twice daily and alfalfa roll (*ad libitum*). Dietary ingredients and nutrient composition data are shown in Table 1 (supplementary material).

Experiment 2: it was held with 80 6-month-old cross-cull cows (Aberdeen Angus – Hereford). Animals started the assay at 9-12 months old, with an initial weight between 427kg to 456kg. Animals were weighed and divided into two groups, 40 animals each. The dietary treatments were used for 22 days and designed as T1- control: the animals received the basal diet without probiotic additive; T2 – probiotic: the animals received the same diet with the addition of *S. boulardii* (5x10^{11} CFU/T). The animals were fed with forage sorghum; 2kg ground corn (with and without probiotic additive) once a day and water *ad libitum*. The nutritional characteristics of the diet are shown in Table 2

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(supplementary material). Table 3 shows supplementary material with a health plan applied to animals from each experiment according to the farm protocol.

2.3. Productive parameter determination

Daily dry matter intake (DDMI) was estimated per pen from the daily supply of feed. Then, the daily intake of the groups 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.

$$\text{Daily weight gain (DWG)} = \frac{Weight\ gain\ (WG)}{Days}$$

$$\text{Feed conversion Index (FCI)} = \frac{Daily\ dry\ matter\ intake\ (DDMI)\ (kg)}{Daily\ weight\ gain\ (DWG)}$$

2.4. Statistical analysis

The WG, DWG, and FCI 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) ($P<0.001$).

3. RESULTS

Table 1 shows the productive parameters of weaned calves during the experimental period (35 days). WG showed significant differences between the control and probiotic treatments ($P\leq0.001$), with a WG of approximately 5 times higher. Similarly, DWG was higher in the probiotic group, while the FCI was lower, indicating a better conversion of feed to weight gain.

Table 1: Productive parameters of weaned calves during the experimental period (35 days)

Treatment	Weight gain (kg)	Daily weight gain (kg)	Intake (kg/DM)	Feed conversion
Control (Group 1)	23.8±5.12a	0.68±0.15a	5.16±0.76	7.85±1.52b
Probiotic (Group 2)	28.8±4.57b	0.82±0.13b	5.16±0.76	6.40±1.15a

Values (Mean±SD) bearing different alphabets in a column differ significantly (P<0.05), according to Fisher's Protected Least Significant Difference Test.

Table 2 shows the productive parameters of cull cows during the experimental period (22 days), where the probiotic treatment resulted in a statistically significant increase in WG and DWG compared to the control treatment ($P\leq0.001$), with an approximate 15 times higher WG. Additionally, the FCI was significantly lower in the probiotic treatment, indicating greater efficiency.

Table 2: Productive parameters of cull cows during the experimental period (22 days)

Treatment	Weight gain (kg)	Daily weight gain (kg)	Feed conversion
Control (Group 1)	34.9±10.3a	1.5±0.5a	6.19±1.98 a
Probiotic (Group 2)	50.1±13.5b	2.5±0.6b	5.25±1.27 b

Values (Mean±SD) bearing different alphabets in a column differ significantly (P<0.05), according to Fisher's Protected Least Significant Difference Test.

Table 3 shows the productive parameters of culling cows during 22 days of the assay according to breed. The obtained WG showed significant differences between groups according to the breed ($P\leq0.001$). Animals in the control treatment had similar WG regardless of breed, but those in the probiotic treatment, particularly those from Aberdeen Angus and Hereford breeds, had significantly higher WG than the control group ($P\leq0.001$).

Weight gain data related to dentition showed that the WG of control animals had no significant differences. However, animals with short-mouth teeth that received probiotics showed WG significantly higher ($P\leq0.001$) than those with broken-mouth (data not shown).

Table 3: Productive parameters of cull cows according to breed and dentition during the experimental period (22 days)

Treatments	Breed	Weight gain (kg)	Dentition	Weight gain (kg)
Control	Crossbreed	40.4±13.6ab	Broken mouth	36.5±4.7a
	Hereford	27.0±6.1a	Short mouth	33.0±4.7a
	Aberdeen-Angus	37.4±5.1ab		
Probiotic	Crossbreed	48.2±16.9bc	Broken mouth	46.0±4.7ab
	Hereford	45.4±8.8bc	Short mouth	54.8±4.7b
	Aberdeen-Angus	56.6±13.8c		

Values (Mean±SD) bearing different alphabets in a column differ significantly (P<0.05), according to Fisher's Protected Least Significant Difference Test.

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4. DISCUSSION

The aim of this study was to evaluate the adaptation and performance of post-weaning calves and cull cows by the inclusion of probiotic *Saccharomyces cerevisiae var. boulardii* RC009 to food as a promising alternative to replace the GPA.

Lactobacillus or *Bacillus* species are commonly used as probiotic supplements for young ruminants targeting the intestine to stabilize the gut microbiota and decrease the risk of pathogen colonization. In contrast, yeasts like *S. boulardii* are known to be effective in adult ruminants and are more suitable for regular feeding practices (Xu et al. 2017).

In this study, the improvement of the WG in calves that received dietary *S. boulardii* RC009 during the 35-day experimental period was shown. These results agree with those of Penha et al. (2011), who reported a greater WG in 18-month-old male cattle fed with probiotics, enzymes, and lactic acid bacteria (amylase, cellulase, protease, lipase, pectinase, *Lactobacillus acidophilus*, *Streptococcus faecium*, *Bifidobacterium thermophilus*, *Bifidobacterium longum*, and zinc) for 75 days compared to cattle that did not receive probiotic supplementation. (Kelsey and Colpoys 2018) showed an improvement in productive parameters in weaned calves fed with a mix of lactic acid bacteria (*Enterococcus faecium, L. acidophilus, L. casei, and L. plantarum)* as probiotics for three weeks. Similarly, Adams et al. (2008) showed an increase in live WG of the Holstein-Friesian bull calves fed with *Propionibacterium jensenii*, (a bacterial strain isolated from raw milk), compared to those that did not receive the probiotic.

On the other hand, Zhang et al. (2016) showed no improvement in average daily gain, dry matter intake, or nutritional digestibility in Holstein calves supplemented with *Lactobacillus plantarum* GF103 and *Bacillus subtilis*. Considering the inclusion of yeast, our results agree with Lesmeister et al. (2004) who demonstrated that *S. cerevisiae* included at 2% in fed of weaned Holstein's calves significantly increased total DDMI. Moreover, DWG improved by 15.6%, compared to the control treatment without probiotics. In our study, supplementation with *S. boulardii* RC009 (5×10^{11} CFU/T) improved the productive parameters in cull cows, Aberdeen Angus showed the highest WG, followed by Hereford and crossbreed. Different results have been reported with the inclusion of yeast in adult ruminant performance. Malik and Bandla (2010) showed that DWG and FCI were higher in calves receiving a probiotic *S. cerevisiae* at a dose of 3×10^9 CFU/T. On the other hand, Vasconcelos et al. (2008) showed no effect of *Lactobacillus acidophilus* (strain NP 51) combined with a single dose of *Propionibacterium freudenreichii* (strain NP 24) on performance and carcass characteristics of finishing beef steers, which could be attributed to the difference in rumen microbial composition. The most commonly marketed products for ruminants are formulated with the yeast cell wall or/and live yeast (Sousa et al. 2018; Cagle et al. 2020; Wang et al. 2023) of *Saccharomyces cerevisiae.* Some authors have shown that daily live yeast supplementation increases average DWG, final weight, and food intake in beef cattle (Maamouri and Ben Salem 2021). Numerous previous studies demonstrated that probiotic inclusion in food ruminants also increased their productive performance (Frizzo et al. 2008; Arowolo and He 2018).

Colombo et al. (2021) in a recent study suggested that the supplementation of a yeast-derived prebiotic and a bacterium as *B. subtilis* improved health conditions and overall productivity during the feedlot receiving period.

In conclusion, the overall benefits of *S. cerevisiae*-based products on animal performance may be linked to improving cattle intestinal health with increased nutrient digestibility (Batista et al. 2022). In our study, we demonstrated the same benefits on performance using *S. cerevisiae var. boulardii* RC009-based products.

Supplementing animal diets with yeast probiotics has been shown to improve productivity in both lactating and growing animals. Certain strains used as active dry yeast have been particularly effective at stabilizing ruminal pH by stimulating certain populations of ciliate protozoa, creating a less acidic ruminal environment that benefits cellulolytic microorganisms' growth and fiber-degrading activities. Yeast cells provide growth factors, such as organic acids and oligosaccharides, B vitamins, and amino acids, which stimulate microbial growth in the rumen and indirectly stabilize ruminal pH (Xu et al. 2017). Thus, yeasts not only act as probiotics but also support the growth of other rumen microorganisms, serving as a prebiotic product.

5. Conclusion

This is the first time that a *S. boulardii* RC009-based product has been shown to be effective in improving productive parameters in both, calves subjected to wean stress and cull cows. A positive impact on the reduction of costs related to animal feeding can be expected since an increase in DWG was correlated with an improvement in FCI.

Authors' Contributions: MVC, PP, SW, and MEO: Methodology and software experiments, formal analysis, and visualization. HRL and PP: Conceptualization and validation. APM: Investigation and writing of the manuscript. LC:

Conceptualization, data curation, writing of the manuscript, supervision, and validation. All authors read and approved the final manuscript.

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Competing of Interests Declaration

The authors have declared no conflict of interest.

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