

# Effect of *Saccharomyces cerevisiae* in a culture or lysate on innate immunity and ruminal and intestinal morphology of steers finished in feedlot

## Efeito da *Saccharomyces cerevisiae* em cultura ou lisada na imunidade inata, na morfologia ruminal e intestinal de novilhos terminados em confinamento

Bruna Fernanda Zdepski<sup>1</sup>; Jayme Augusto Peres<sup>2</sup>; Bianca Milla<sup>3</sup>; Sarah Naiverth de Oliveira<sup>3</sup>; Carolina Rodrigues Depaoli<sup>3</sup>; Ana Carolina Araujo Abreu<sup>3</sup>; Gabriela Garbossa<sup>1</sup>; Ricardo Pereira Manzano<sup>4</sup>; Heloisa Godoi Bertagnon<sup>2\*</sup>

### Highlights

*S. cerevisiae* increased oxidative metabolism and decreased inflammation.  
Culture of yeast attenuated the ruminal papilla alteration compatible with SARA.  
Autolyzed *S. cerevisiae* increased villus crypt area of duodenum and jejunum.  
Both classes of *S. cerevisiae* minimized the occurrence BRD in feedlot steers.

### Abstract

The objective of this study was to evaluate whether the administration of two classes of *Saccharomyces cerevisiae* improved the health of feedlot-finished steers. A total of 33 Angus-Nellore blood ½ steers (body weight of 350 ± 10 kg and 11 ± 5 months old) fed an energy diet were evaluated at the beginning (0 and 16 days), middle (44 and 72 days), and end (100) of the feedlot based on the inflammatory status and health of the respiratory and digestive tracts. Inflammation and innate immunity were measured using leukocyte oxidative metabolism (OM), serum haptoglobin levels, and the neutrophil-to-lymphocyte ratio. Respiratory health was verified based on nasal secretion, rectal temperature, and lung lesions during slaughter. The digestive health was verified by histomorphology of the rumen and intestine. The treatments were as follows: *Saccharomyces cerevisiae* in culture (CUL n=12), *S. cerevisiae* in the autolyzed form (AUT n=10), and control diet (CON, n=11). In the CUL group, OM increased by 7% on average, with lower indicators of respiratory disease and inflammatory status in the intermediate and

<sup>1</sup> Master Post-Graduate, Program of Veterinary Science, Universidade Estadual do Centro Oeste, UNICENTRO, Guarapuava, PR, Brazil. E-mail: brunazdepski@hotmail.com; gabigarbossa@hotmail.com

<sup>2</sup> Profs. Drs. of Course of Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail perespatologia@gmail.com; hbertagnon@unicentro.br

<sup>3</sup> Students of Graduate of Course of Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail: biancamilla36@gmail.com; naiverths@gmail.com; depaolicarolina@yahoo.com; anacarolinaaabreu@gmail.com

<sup>4</sup> Consultant and owner of Nutripec Consultoria, Catanduva, São Paulo, SP, Brazil. E-mail: rpmanzan@yahoo.com.br

\* Author for correspondence

final stages of the feedlots. The ruminal papillae of these animals were 30% thinner than those of the CON group and had lower ruminitis scores. Although AUT promoted a 10% increase in OM and a lower inflammatory status in the intermediate and final stages of the feedlot, steers showed more indicators of respiratory diseases than those in the CUL group. The duodenal villus area and jejunal villus height in the AUT group still increased by 25 and 15%, respectively. Its effect on the ruminal papilla was subtle, only decreasing the ruminitis score. We concluded that both groups of *S. cerevisiae* improved the health of animals compared with the control group, indicating that supplementation with *S. cerevisiae* cultures resulted in the decreased alteration of rumen papillae, compatible with a lower occurrence of subclinical ruminal acidosis syndrome (SARA), reflecting immunological gains and a lower occurrence of bovine respiratory disease (BRD). The *S. cerevisiae* autolysate showed a higher intestinal effect and lower activity in the rumen papillae. Although this treatment showed an immunological gain similar to a yeast culture, it reflected the lower health of the respiratory tract.

**Key words:** Bovine respiratory disease (BRD). Inflammation. Reactive oxygen species (ROS). Subacute ruminal acidosis syndrome (SARA). Yeast.

## Resumo

O objetivo do trabalho foi avaliar se a administração de duas classes de *Saccharomyces cerevisiae* melhoram a saúde de novilhos terminados confinados. Um total de 33 novilhos ½ sangue Angus-Nelore, (350 ± 10 kg peso vivo e 11 ± 5 meses de vida) alimentados com dieta energética foram avaliados no início (0 e 16 dias), meio (44 e 72 dias) e final (100) de confinamento, por meio de indicadores de inflamação e saúde do trato respiratório e digestório. A inflamação e imunidade inata foram aferidas por metabolismo oxidativo de leucócitos (MO); teores séricos de haptoglobina e razão neutrófilo:linfócito. A saúde respiratória foi verificada por secreção nasal, temperatura retal e lesões pulmonares no abate. E a saúde digestória foi verificada por histomorfologia do rúmen e intestino. Os tratamentos foram: *Saccharomyces cerevisiae* na forma de cultura (CUL n=12), *S. cerevisiae* na forma autolisada (AUT n=10) e dieta controle (CON n=11). O CUL apresentaram incremento médio de 7% no MO, menores indicadores de doença respiratória e de status inflamatório na fase intermediária e final do confinamento. As papilas ruminais destes animais era 30% mais delgada que o CON, e apresentavam menor escore de ruminite. Apesar do AUT promover incremento de 10% no MO e menor status inflamatório na fase intermediária e final do confinamento também, os novilhos apresentaram mais indicadores de doenças respiratórias que o CUL. O AUT ainda aumentou 25% da área de vilosidade de duodeno, e 15% na altura da vilosidade jejunal. Sua ação em papila ruminal foi tênue, minimizando apenas o escore de ruminite. Concluiu-se que ambas as classes de *S. cerevisiae* aumentaram a saúde dos animais em relação ao CON, destacando-se a suplementação com cultura de *S. cerevisiae*, que resultou em menor alteração de papilas ruminais compatíveis com menor ocorrência de SARA, melhores resultados do sistema imunológico e menor ocorrência de CRB. O autolisado de *S. cerevisiae* demonstrou maior efeito intestinal, com atuação de menor impacto em papila ruminal. E apesar de ter ganho imunológico similar ao encontrado ao CUL, apresentou menor influência na saúde do trato respiratório.

**Palavras-chave:** Complexo respiratório bovino (CRB). Espécies reativas de oxigênio (ERO). Inflamação. levedura. Síndrome acidose ruminal subaguda (SARA).

## Introduction

Feedlot-finished steers are essential for the intensification of meat production systems but they present important health challenges. The animals are fed a high-energy diet that caused subclinical ruminal acidosis syndrome (SARA). A daily drop in ruminal pH promotes ruminitis, which impairs ruminal absorption and permeability. These changes promote an inflammatory state that decreases immunity in animals (Minami et al., 2021).

Both SARA and the intrinsic stress of the feedlot system (transport, segregation of lots, and changes in the environment) weaken the immune system, resulting in the occurrence of infectious diseases, such as bovine respiratory disease (BRD) complex, with up to 69% morbidity and 15% mortality in feedlots (Estima-Silva et al., 2020; Heidmann et al., 2021).

The use of yeast as a food additive for livestock improves animal performance and mitigates the undesirable effects of animal management systems. These additives act as modulators of ruminal fermentation and mycotoxin adsorbents and can improve the intestinal and immune health. All these responses are dependent on the type of feed, production, and challenges experienced by animals, as well as the classes of yeast used as additives (Zeoula et al., 2008; Broadway et al., 2015).

*Saccharomyces cerevisiae* is the most commonly used yeast in animal feed and can be processed according to the objective expected in animals. Doubts still exist regarding its mode of action and influence on bovine performance because

the different forms of yeast processing influence the characteristics and availability of yeast (Broadway et al., 2015; Uyeno et al., 2015).

Yeast cultures are produced by a controlled fermentation process, generating beneficial metabolites for the ruminal microbiota related to the metabolic pathways of amino acids, carbohydrates, and volatile fatty acids, which increase the ruminal microbial population. Additionally, these metabolites serve as nutrients for anaerobic fibrolytic and lactic acid bacteria in the rumen. This selection of microbiota contributes to the stability of ruminal pH, which reduces SARA levels, and consequently improves ruminal health (Uyeno et al., 2015; Diaz & Branco, 2019).

In the autolyzed class, a disruption of the cell wall was observed owing to the greater abundance of the cytoplasmic content and cell wall than in intact yeast cells. The components of the cell wall (mannanoglycosaccharides (MOS) and  $\beta$ -glucans) have an immunomodulatory effect. They are absorbed by the gastrointestinal tract and stimulate the activity of phagocytes and B lymphocytes as well as cellular communication in the body, which reduces infections in challenged tissues by increasing the immune efficiency without inducing an exaggerated inflammatory response. Additionally, pathogenic bacteria, toxins, and viruses are linked and carried out in the intestinal lumen and increase the phagocytic activity, especially in macrophages (Shurson, 2018). The cytoplasm is rich in B complex vitamins (B1, B2, B6, pantothenic acid, niacin folic acid, and biotin), which are essential for the maintenance of metabolism, energy

production, differentiation, and cell growth. They also contain essential minerals, proteins, and nucleic acids that serve as nutrients for animals (Queiroz et al., 2015).

Although several studies have demonstrated the efficiency of different yeast classes in the performance of feedlot ruminants (Mao et al., 2013; Stadler et al., 2019), few have exclusively focused on immunity (Virmond et al., 2020; Garbossa et al., 2023), reduction of inflammation of the gastrointestinal tract (Diaz et al., 2018; Xiao et al., 2016), and reduction of BRD (Finck et al., 2014; Virmond et al., 2020; Garbossa et al., 2023). Additionally, factors such as age, stress, type and adaptation to the diet, and the class of yeast products interfered with these results. Therefore, each type of management, with its particularities and the concentration and class of used yeasts, should specifically be analyzed for each case (Broadway et al., 2015; Uyeno et al., 2015).

Thus, the objective of this experiment was to evaluate the effect of products derived from *S. cerevisiae*, such as the culture or autolysis, on the morphology of the ruminal papilla, innate immunity, and consequently on the occurrence of BRD in feedlot-finished steers that received an energy diet of 60% concentrate and 40% corn silage.

## Materials and Methods

This experiment was approved by CEUA-UNICENTRO, protocol 11/2020 on 08/05/2020.

### *Experimental facilities*

The experiment was conducted in the didactic unit, research, and extension of beef cattle (NUPRAN) of the Veterinary Sciences Master of UNICENTRO, located in Guarapuava, Paraná, Brazil. The climate of the region is humid mesothermal subtropical (CFB) without a dry season and with cool summers and moderate winters. According to the Köppen classification, Guarapuava has an altitude of 1,100 m, with an average annual precipitation of 1,944 mm, minimum annual average temperature of 12.7 °C, maximum annual average of 23.5 °C, and relative humidity of 77.9%. During the experiment, the ambient temperatures ranged from 9 to 20 °C and the relative humidity ranged from 80 to 75%. Approximately 70 days after the start of the experiment, the ambient temperatures abruptly changed from 18 to 9 °C.

### *Experimental design*

Thirty-three whole Angus-Nellore blood ½ steers were used, with an initial average weight of 350 ± 10 kg and initial average age of 11 ± 0.5 months. The animals were previously dewormed and had not been vaccinated against BRD. The experimental design was completely randomized, with three treatments evaluated at five time points, wherein each animal was an experimental unit. The steers in the CON group were fed a yeast-free diet (control, n = 11), those in the CUL were fed a diet containing a culture of *S. cerevisiae* (Cultron - Aleris, São Paulo, Brazil, n = 12), and those in the AUT group were fed a diet containing autolyzed *S. cerevisiae* (Cultron Pro - Aleris, São Paulo, Brazil) (Table 1).

**Table 1**  
**Chemical composition of *S. cerevisiae* classes used**

Parameter	Culture*	Autolyzed**
Dry matter, % NM	92	94,5
Mineral matter, % DM	4	5,5
Crude protein, % DM	45	32
Ethereal extract, % DM	7	2,5
Crude fibre, % DM	4	1,5
β-glucans, % DM	15-17	20-22
mannan oligosaccharides, % DM	8-10	12-13
K, % DM	0,38	0,70
Ca, % DM	0,05	0,90
P, % DM	0,78	0,70

(\* Culture medium is obtained by fermentation in controlled nutrient medium containing cane molasses and corn derivatives, containing fermentation metabolites containing leucine, valine and isoleucine, vitamins, enzymes and organic acids, commercial product Cultron - Aleris®, São Paulo, Brazil \*\* the autolyzed class still contains the cytoplasmic constituents, commercial product Cultron Pro - Aleris®, São Paulo, Brasil).

The animals were obtained from a commercial property located approximately 50 km from the experimental site. They were allocated semi-covered stalls with an area of 7 m<sup>2</sup> per animal with a concrete floor and wood separation between stalls. The stalls were cleaned daily by manually removing feces. Each stall contained a concrete feeder and automatic drinker. After 15 days of acclimatization to the site, the experiment was started and this period was classified as day 0 (D0), wherein the additives were administered and the property diet (60% corn silage and 40% concentrate) was gradually changed to 40% corn silage and 60% concentrate for 16 days (there was a change of 2% of silage and concentrate every 2 days). Analyses were conducted at the beginning (D0 and D16), middle (D44

and D72), and end of the feedlot (D100). The animals were slaughtered on D105.

Feed was provided *ad libitum*, with 5% of leftovers (Table 2). The concentrates used were soybean meal, soybean hulls, barley radicle, milled corn grains, milled barley grains, corn germ, calcitic limestone, dicalcium phosphate, common salt, livestock urea, and a vitamin-mineral mix. The voluntary consumption of food was recorded daily by weighing the offered quantity and leftovers of the previous day, considering the adjustment of daily consumption, to maintain the leftovers at 5% of dry matter (DM). The additives were diluted and homogenized in 50 g of concentrate and added to the diet at the time of each feed. The control group received only 50 g of the concentrated meal.

**Table 2**  
**Chemical composition of the feed used for animal feeding and average values of the experimental ration, based on total dry matter (DM)**

Parameter	Corn silage *	Concentrate	Experimental diet*
Dry matter, % MN	34,22	91,97	63,10
Mineral matter, % DM	2,64	6,36	4,50
Crude protein, % DM	6,57	20,20	13,39
Ethereal extract, % DMS	2,43	2,05	2,24
Neutral detergent fibre, % DM	45,30	31,47	38,39
Acid detergent fibre, % DM	26,57	13,08	19,83
Lignin, % DM	3,85	4,73	4,29
Total digestible nutrientes, % DM	69,24	78,68	73,96
Ca, % DM	0,13	1,67	0,90
P, % DM	0,25	0,58	0,42

Premix guaranteed level per kg of concentrate: vit. A = 16000IU, vit D3 = 2000IU, vit. E = 25 IU, S = 0.36g, Mg = 0.74g, Na = 3.6g, Co = 0.52mg, Cu = 22.01mg, F = 18.00mg, I = 1.07mg, Mn = 72.80mg, Se = 0.64mg, Zn = 95.20mg.

### *Experimental sample*

The innate immunity was evaluated by measuring the leukocyte oxidative metabolism. The inflammatory status was evaluated using a leukogram, serum haptoglobin dosage, and rectal temperature. Respiratory disease indicators were evaluated based on the presence of mucopurulent nasal secretions. All samples were analyzed at the beginning (D0 and D16), middle (D44 and D72), and final (D100) feedlot periods. At the time of slaughter, we evaluated pneumonic lesions and histological changes in the rumen and small intestine.

Blood samples were collected from each animal through external jugular venopuncture in 3 vacuum tubes. The leukocyte oxidative metabolism was measured by the quantitative technique of nitroblue tetrazolium (NBT) (Flores et al., 2019) in the blood samples collected with heparin tubes (4 ml of blood). In tubes

with ethylenediamine tetraacetic acid (EDTA; 4 ml of blood), the leukogram was conducted using an automatic cell counter (SDH-3 VET, Labtest, São Paulo, Brazil), and cell populations were classified into blood smears according to morphotintorial characteristics through optical microscopy as neutrophils, lymphocytes, monocytes, eosinophils, and basophils. We calculated the neutrophil-to-lymphocyte ratio. The serum haptoglobin level was measured in blood samples collected without anticoagulants (8 ml of blood). The blood serum was separated from the whole blood by centrifugation at 3500 rpm for 15 min and frozen at -20 °C until the end of collection. Haptoglobin was measured using a commercial ELISA kit (Bovine Haptoglobin EB0011 Fine Test, WU, China) according to the manufacturer's instructions.

Rectal temperature was measured using digital thermometry. The mucopurulent nasal secretion was observed during the four

days of each experimental period (two days before and after the aforementioned days), always 20 min before the animals' tract. The four evaluations for each period were transformed into the average frequency of animals with mucopurulent nasal secretions, and statistical analysis was conducted.

On the day of slaughter, lung, rumen, duodenum, and jejunum fragments were collected. Pulmonary fragments with an area of approximately 2 cm<sup>2</sup> was collected from each animal, where a transitional area was present between normal tissue and consolidation lesions. Normal tissue fragments were collected from the ventral cranial lobe in the absence of macroscopic lesions. The tissues were fixed in 10% formaldehyde for 48 h and embedded in paraffin, sectioned to make histopathological slides, stained with hematoxylin and eosin (HE), and observed under an optical microscope. According to Ceribasi et al. (2014), fragments were classified according to the absence or presence of pneumonia.

Fragments of the ventral sac of the rumen (5 cm<sup>2</sup>), duodenum, and jejunum (1 cm<sup>2</sup>) were collected and stored in 70% alcohol. They were embedded, cut, and stained with HE. Rumen: the thicknesses of the papilla, keratin layer of the rumen epithelium, connective tissue, and non-keratinized epithelium were measured in 15 papillae of each animal. The mitotic index (MI) of the nuclei of the basal layer of the ruminal epithelium and degree of neutrophilic infiltration in the ruminal epithelium were scored from 1 to 3. A score of 3 indicated the highest degree of MI and inflammatory cell infiltration, as described by Diaz et al. (2018). The small intestine villus height, crypt depth, and villus:crypt ratio were measured

in 10 villi per animal. All histological analyses were conducted using a Leica Qwin Image Analyzer equipped with a Leica light electron microscope. Images of each cut were captured using objective lenses at 40x magnification and scanned using a camera for morphometric analysis.

### *Statistical analysis*

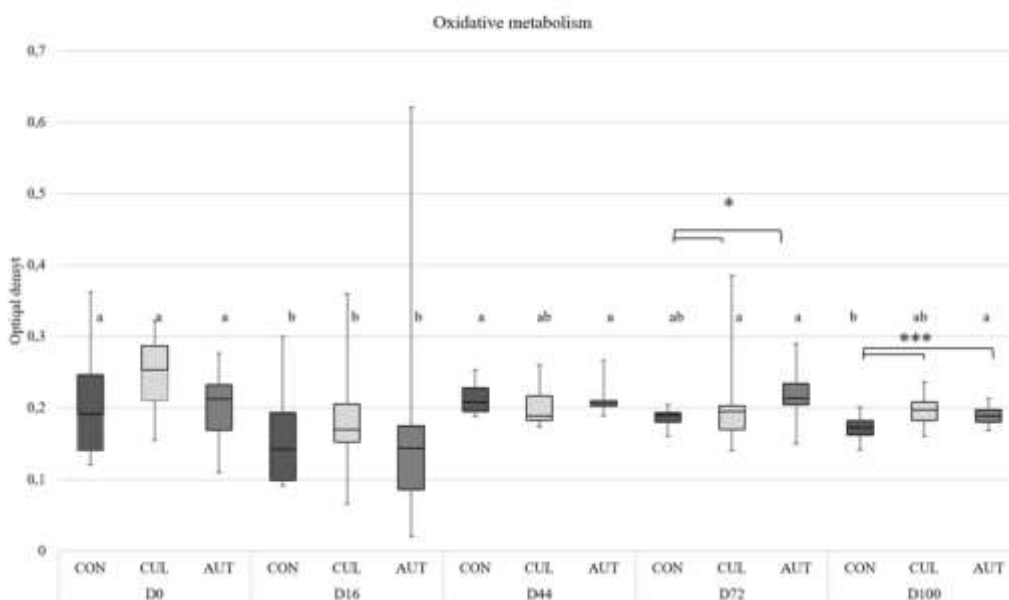
Statistical analyses were conducted using the InStat GraphPad statistical software. The data were evaluated for normality (Kolmogorov-Smirnov test) and homogeneity (Barelett tests). All data passed the normality test; however, data of the leukocyte oxidative metabolism and serum haptoglobin levels were not homogeneous. They were treated as non-parametric data and presented as medians and first and third quartiles. The occurrence of mucopurulent nasal secretion, pneumonia, ruminitis, and ruminal papillary MI scores were analyzed using the chi-square test and presented as the frequency. Other data were evaluated using parametric tests. For the parameter analyses of ordinal quantities, means or medians were compared. If a statistical difference was found, we proceeded with the time analysis; each treatment was analyzed over time by repeated analysis of variance (ANOVA; parametric or non-parametric) and Tukey or Dunn's test to verify the period in which the treatment began to exert its effect. The treatment effect was also analyzed by comparing the different treatments in each period using non-repeated ANOVA (parametric or nonparametric) and Tukey or Dunn post hoc tests to verify whether the treatment influenced the variable in each period. Statistical significance was set at  $P < 0.05$ .

## Results and Discussion

### *Oxidative metabolism and inflammatory status*

Data on the oxidative metabolism of blood leukocytes of feedlot-finished steers are shown in Figure 1. Regarding the effect of time, on D16, steers in all groups showed a reduction in oxidative metabolism, and

only those in the CON group showed this reduction on D100 (CON: D16 and D 100 < D0, D44, and D72, P=0.03; CUL: D16<D0, P=0.001 and AUT D16< D0, D44, D72, and D100; P=0.004). Regarding the treatment effect, steers in the CUL and AUT groups showed 7 and 10% increases in oxidative metabolism compared with those in the CON group on D72 and D100, respectively (P=0.02 and P=0.0009).



**Figure 1.** Oxidative metabolism of blood leukocytes of feedlot-finished steers subjected to culture or autolyzed of *Saccharomyces cerevisiae*.

Values expressed in median first and third quartiles and maximum and minimum values. CON- Control, CUL- culture of *S. cerevisiae*, AUT- autolyzed *S. cerevisiae*. Different lowercase letters in the same treatment indicate statistical difference for the time effect (P<0,05);

\*P<0,05, \*\* P<0,001 indicate statistical difference for the treatment effect.

The inflammatory status (neutrophils lymphocytes ratio, serum haptoglobin levels, and rectal temperatures) are shown in Figure 2. Total leukocyte, lymphocyte, eosinophil, basophil, and monocyte counts showed no

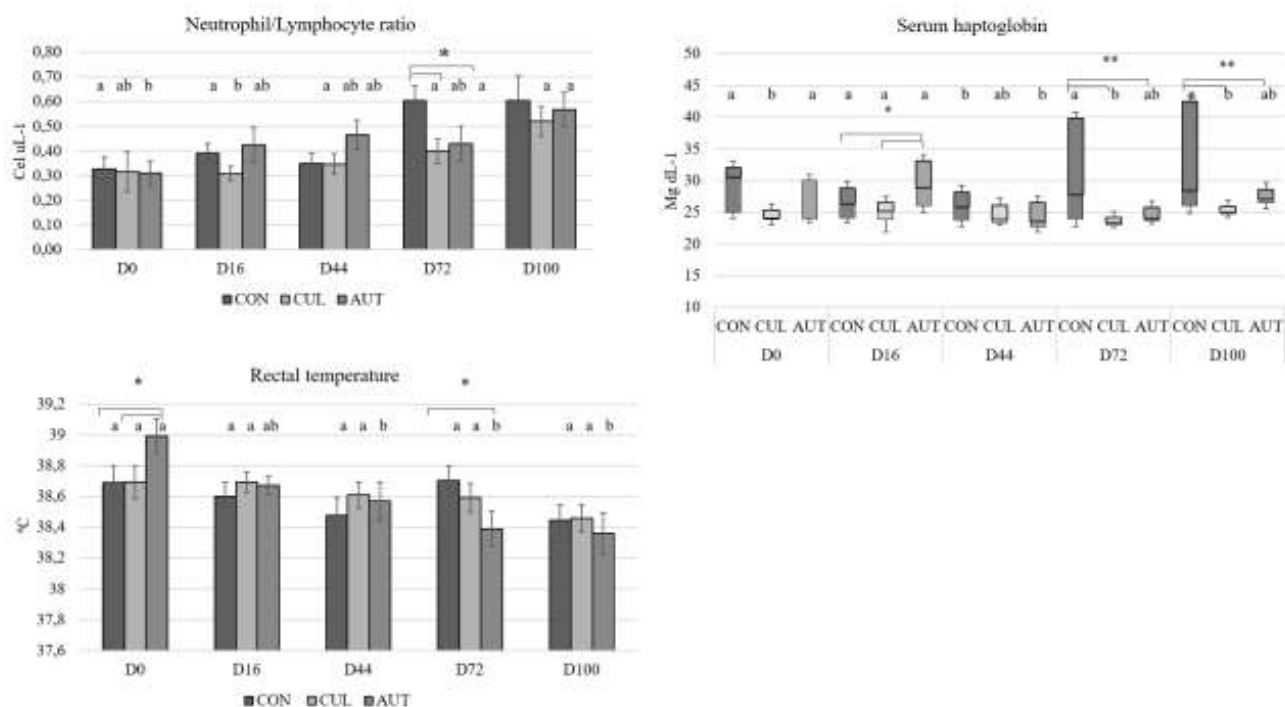
statistical differences (Supplementary Data). Most animals presented with leukocytosis, neutrophilia, and lymphocytosis at all time points. We observed an increase in neutrophils and the neutrophil:lymphocyte



ratio on D72 in the CON group owing to the treatment effect ( $P=0.04$  and  $0.05$ ), without a time effect. The steers in the CUL group showed a higher neutrophil:lymphocyte ratio on D100 than on D16 ( $P=0.04$ ), and the AUT presented a higher neutrophil:lymphocyte ratio on D100 than on D0 ( $P=0.03$ ).

Both serum haptoglobin level and rectal temperature were higher in the AUT group in the initial feedlot periods than those in the other groups, during either the comparison between periods or comparison

between treatments (serum haptoglobin-AUT-D0 and D16> D44  $P=0.0001$ , D16 AUT > CON and CUL  $P=0.05$ ; rectal temperature, D0> D44, D72 and D100  $P=0.001$  D0 AUT > CON and CUL  $P=0.05$ ). In the intermediate and final feedlot periods, serum haptoglobin levels in the steers of the CON group were higher than those of the other groups (CON> CUL and AUT on D72 and on D100  $P=0.02$ ;  $P=0.04$ ), whereas only the rectal temperature of the steers of the CON group was higher than that of the AUT group on D72 ( $P=0.01$ ).



**Figure 2.** Inflammatory status of feedlot-finished steers subjected to culture or autolyzed of *Saccharomyces cerevisiae*.

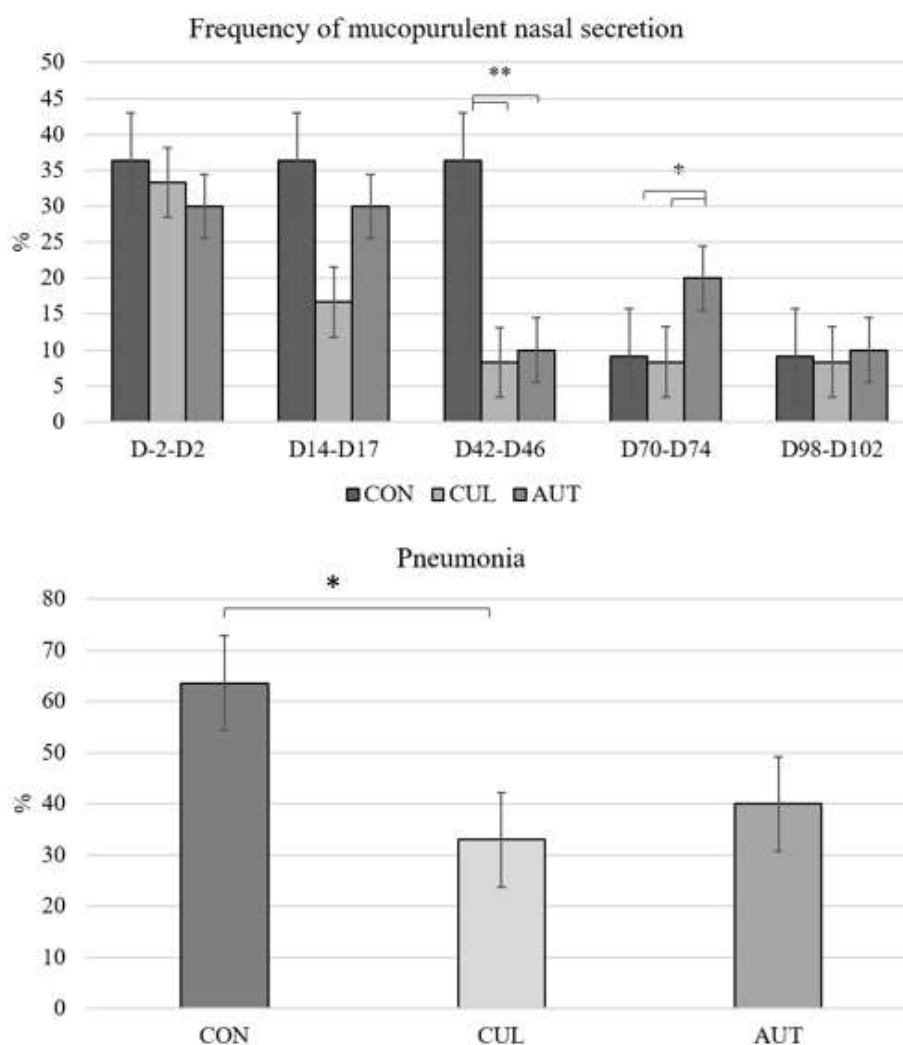
Neutrophils and lymphocytes ratio and rectal temperatures- Values expressed as mean and standard deviation, Serum haptoglobin levels- Values expressed in median first and third quartiles and maximum and minimum values. CON- Control, CUL- culture of *S. cerevisiae*, AUT- autolyzed *S. cerevisiae*. Different lowercase letters in the same treatment indicate statistical difference for the time effect ( $P<0.05$ );

\*  $P<0.05$ , \*\*  $P<0.001$  indicate statistical difference for the treatment effect.

*BRD Indicators*

Mucopurulent nasal secretion data and pneumonia occurrence (identified by pulmonary histopathological analysis on the day of slaughter) are shown in Figure 3. The frequency of mucopurulent nasal secretions decreased over time, most rapidly in the CUL group, followed by the AUT group and finally, the CON group. On D42-D46, more animals

in the CON group presented mucopurulent nasal secretions than in the other groups ( $P = 0.001$ ), and on D70-D74, these secretions were higher in the animals of the AUT group than in the other groups ( $P = 0.05$ ). The frequency of pneumonia was higher in the CON group than in the CUL ( $P=0.002$ ) and AUT ( $P=0.08$ ) groups. No difference was observed in the pneumonia frequency between the yeast treatments ( $P=0.44$ ).



**Figure 3.** Frequency of mucopurulent nasal secretions and of pneumonia of feedlot-finished steers subjected to culture or autolyzed of *Saccharomyces cerevisiae*. CON- Control, CUL- culture of *S. cerevisiae*, AUT- autolyzed *S. cerevisiae* \*  $P < 0,05$ , \*\*  $P < 0,001$  indicate statistical difference for the treatment effect.

### *Rumen, duodenum, and jejunum histopathological data*

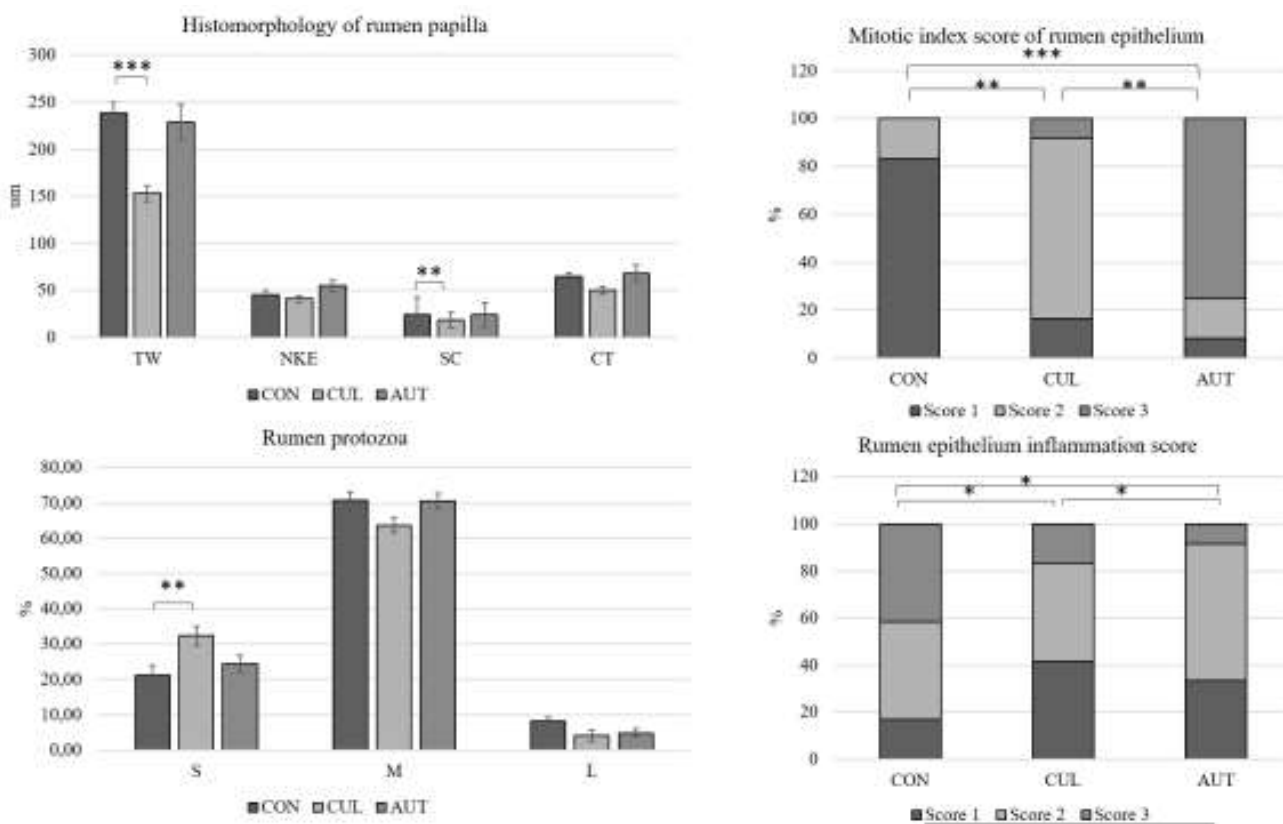
The histopathological evaluation of the rumen and ruminal protozoa is shown in Figure 4. Ruminal papilla thickness in the CUL group was approximately 30% lower than that in the CON and AUT groups. The corneum stratum and total papilla width in the CUL group were 25 and 36% lower than those in the CON and AUT ( $P = 0.005$ ,  $P = 0.0001$ ) groups, respectively, whereas the thicknesses of the non-keratinized epithelium and connective tissue were unaffected by the treatments. Considering the MI and degree of ruminal epithelium inflammation, all groups were different from each other, with lower MI in the CON group and intermediate MI in the CUL group compared with that in AUT ( $P=0.002$ ). Ruminal inflammation in the CON group was higher than that in the CUL group ( $P=0.04$ ).

Regarding the intestinal absorptive capacity, the duodenal villus and jejunal villi in the AUT group increased by 30 and 15%, respectively, compared with those in the other groups ( $P=0.004$  and  $0.001$ , respectively). The villus:crypt ratio in the duodenum was 25% higher in the AUT group ( $P=0.003$ ) than in the other groups. The duodenal crypt depth in the CON group was 17% higher than that in the other groups ( $P=0.03$ ), without differences in other variables (Figure 5).

The *S. cerevisiae* culture promoted better animal health as its main action was to reduce inflammation of the ruminal papilla, as observed by lower neutrophilic infiltration, lower corneum stratum thickness, and lower

ruminal papillae width. These histological results indicated a lower occurrence of SARA. Additionally, this treatment promoted a lower inflammatory status in the steers, as confirmed by lower serum haptoglobin levels and lower neutrophil:lymphocyte ratios than those in the CON group. An improved immune capacity, demonstrated by higher leukocyte oxidative metabolism and consequently, lower occurrence of diseases, such as BRD, indicates that this class of yeast can be used as a preventive measure against inflammatory and infectious conditions.

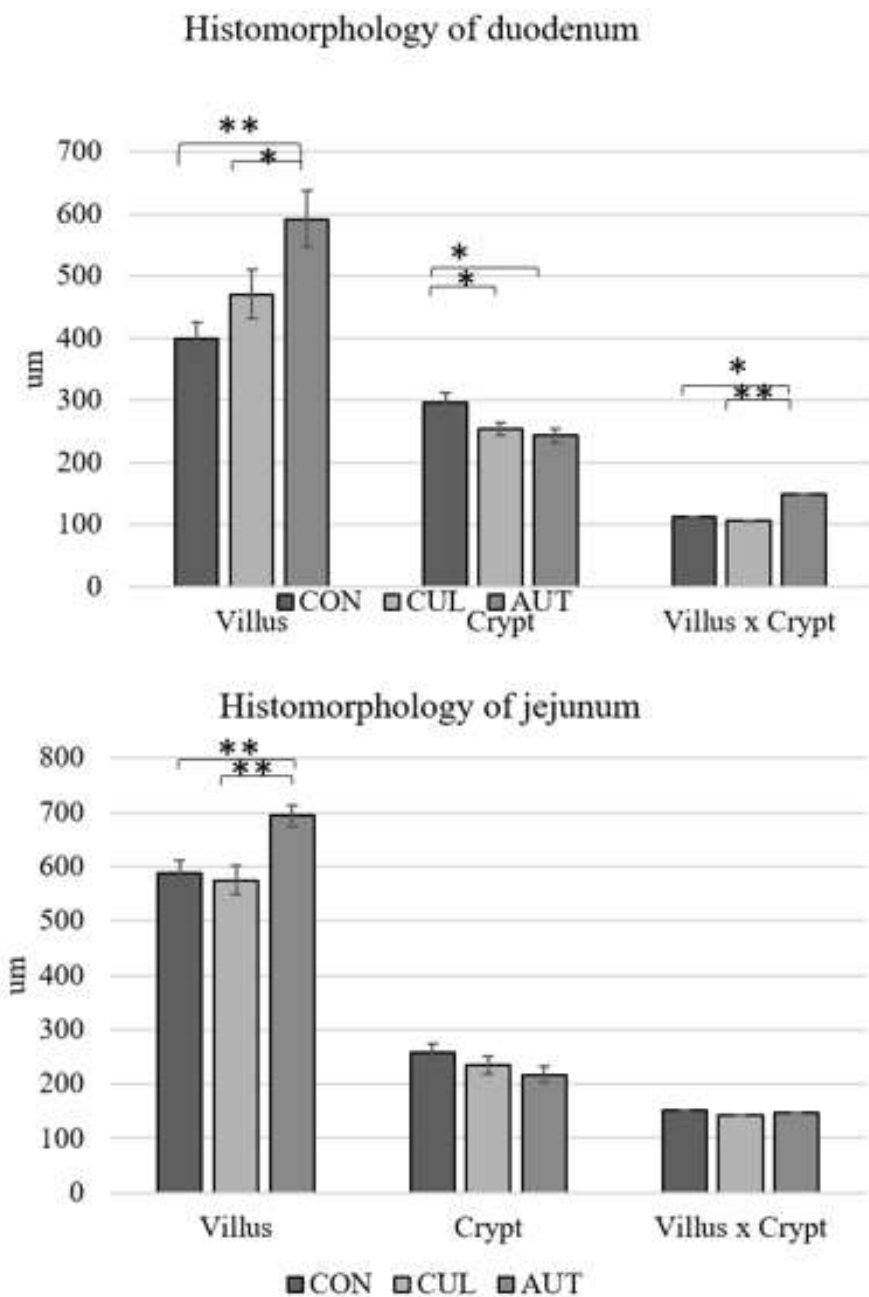
In contrast, the lysate of *S. cerevisiae* mainly acted in the small intestine, and although it attenuated ruminal papillae inflammation, this action showed a lower intensity than that in the CUL group. The inflammation in the AUT group was still higher than that in the CUL and CON groups on D0 and D16, indicating that the animals in the AUT group already had initial inflammation. Despite this, this class of yeast promoted positive effects on the leukocyte oxidative metabolism, which decreased the inflammatory status during the intermediate and final feedlot periods. Although these steers showed a higher frequency of mucopurulent nasal secretion on D72 than those in the other groups, they presented the same frequency of pneumonic lesions on the day of slaughter, similar to those in the other two treatments. This indicates that this class of yeast can be used in animals with inflammatory conditions or in healthy animals as it helps restore health and prevent new inflammation and infections.



**Figure 4.** Histopathological evaluation of the rumen and ruminal protozoa of feedlot-finished steers subjected to culture or autolyzed of *Saccharomyces cerevisiae*. Legend: CON- Control, CUL- culture of *S. cerevisiae*, AUT- autolyzed *S. cerevisiae*. TW- total papilla width, NKE- non-keratinized epithelium, SC- corneum stratum and iextrato córneo, CT- connective tissue. S- small (up to 40 x 60 µm), M- Medium (between 100 x 150 µm), L-large (upon to 150 µm). \* P<0,05, \*\* P<0,001 , \*\*\* P<0,0001 001indicate statistical difference for the treatment effect.

The feedlot system was challenging for animals as most steers presented leukocytosis by neutrophilia and lymphocytosis at all periods, according to the reference values published by Tizard (2014). The stress caused by sample collection may have caused this phenomenon, as observed by Garbossa et al. (2023) and Virmond et

al. (2020). The stress is more intense in the initial periods of the feedlot, with greater cortisol secretion, which is responsible for the decrease in immune cell activity, such as neutrophils (Tizard, 2014; Estima-Silva et al., 2020); all groups showed a reduction in leukocyte oxidative metabolism in the early periods of the feedlot.



**Figure 5.** Histopathological evaluation of the duodenum, and jejunum of feedlot-finished steers subjected to culture or autolyzed of *Saccharomyces cerevisiae*. Legend: CON- Control, CUL- culture of *S. cerevisiae*, AUT- autolyzed *S. cerevisiae*. \* P<0,05, \*\* P<0,001 , \*\*\* P<0,0001 001 indicate statistical difference for the treatment effect.

Oxidative metabolism or leukocyte respiratory explosion is the formation of intracellular reactive oxygen species (ROS) in phagocytes with a bactericidal function. Neutrophils are the main blood phagocytes responsible for the first nonspecific line of defense. A decrease in their function increases susceptibility to infectious diseases (Tizard, 2014). As no reference value parameter was available for this variable, the data were compared with each other, as in the analyses conducted by Garbossa et al. (2023) and Virmond et al. (2020). Thus, on D16, the three treatments presented this reduced parameter, justifying the fact that most animals presented a mucopurulent nasal secretion at the beginning of the feedlot, a period with the highest incidence of BRD in feedlot cattle, as indicated by Heidmann et al. (2021).

Bovine inflammatory status can be identified with the occurrence of neutrophilia (above 5000 cells  $\text{mm}^{-3}$ ), changes in the neutrophil:lymphocyte ratio (below 0.1 or above 0.8), high rectal temperature (above 39.5 °C), or increase in the haptoglobin serum level (above 20 mg/dL) (Tizard, 2014; Malafaia et al., 2016; Sivinski et al., 2022). During the experiment, all inflammatory parameters of the animals in the CON group worsened on D72 and D100. This indicates that the steers in the CON group suffered more intense inflammatory or infectious challenges between the middle and end of the feedlot period. On D72, we observed a decrease in the ambient temperature; however, these steers did not present any clinical indicators of respiratory diseases. *S. cerevisiae* products mainly act on the gastrointestinal tract (Broadway et al., 2015); thus, we hypothesized that the

inflammation/infection in the CON group mainly occurred with SARA, especially on D72. *S. cerevisiae* supplementation, either as a culture or an autolyzed class, promotes the effects on neutrophils after 40 to 50 days of supplementation (Garbossa et al., 2023; Virmond et al., 2020). We believed that the challenge of the diet occurred for all animals, but the animals in treated groups were better able to maintain homeostasis, either by greater immune efficiency, by the lower incidence of digestive disorders, or by the association of both, than those in the control group.

Serum haptoglobin is an earlier inflammatory marker than leukogram. It is an acute-phase protein synthesized by hepatocytes prior to leukocyte response. It is secreted approximately 6-8 h after injury and its maximum concentration is reached within 2-5 days (Tothova et al., 2014). Similar to our findings, the results of Sivinski et al. (2022) indicated that supplementation with a culture of *S. cerevisiae* improved the neutrophil function and increased the efficiency of the innate immune response, which was responsible for the decrease in serum haptoglobin levels during the transition period of dairy cows. As the most common conditions in feedlot cattle are BRD and SARA, the health of animals was mainly monitored through the indicators of these diseases (Malafaia et al., 2016).

The animals in the AUT group showed higher levels of serum haptoglobin and rectal temperature than those in the CON group, and a higher frequency of mucopurulent nasal secretions than those in the CUL group in the initial period. These findings support the higher numerical frequency of animals with

pneumonia in the AUT group than in the CUL group during slaughter. This suggests that in the AUT, the animals were sick before *S. cerevisiae* supplementation due to a specific effect. Subclinical BRD is common in feedlots. Magalhães et al. (2017) reported that 81.1% of feedlot steers showed histopathological lesions in the lungs, which were compatible with pneumonia, that were not diagnosed before slaughter.

This difference in the inflammatory marker levels between the AUT and CUL groups can also be explained by the site of action of the yeasts. The culture of *S. cerevisiae* acts in the ruminal environment, favoring the growth of lactic acid-consuming microorganisms (Wagner et al., 2016), which have a positive effect on the stability of ruminal pH and reduce the occurrence of SARA (Broadway et al., 2015; Xiao et al., 2016; Kovács et al., 2020). The pH stability also reduces the lysis of gram-negative bacteria and decreases the release of lipopolysaccharides (LPSs) in the ruminal environment. In SARA, histopathological alterations in the ruminal papillae enable LPSs to reach the hepatic bloodstream, activate the production of pro-inflammatory cytokines and other mediators, and produce an acute-phase response, which promotes immunosuppression (Shen et al., 2019). Although we did not find information in the literature regarding when yeast supplementation initiates its effect on the gastrointestinal tract, its action on ruminal pH buffering is believed to be immediate based on local activity; this differs from immune stimulation, which can be influenced by stress inherent to feedlots (Diaz & Branco, 2019; Heidmann et al., 2021).

The alteration of ruminal papillae caused by SARA is called parakeratosis, which can be identified by an increase in the rumen papillary and corneum strata and a decrease in rumen epithelial integrity. The loss of rumen epithelial integrity enables bacteria and LPSs to penetrate the inner layers of the papillae. It produces inflammation and edema, both of which contribute to papillary thickening. Inflammatory processes decrease the cell mitosis capacity (MI) and reduce ruminal epithelium renewal, as observed in the CON group and at moderate severity in the AUT group (Kovács et al., 2020; Sanches et al., 2020).

The lower corneum stratum thickness, width of the ruminal papilla, ruminal inflammation scores, and intermediate MI in the CUL group indicated a lower occurrence of SARA. These results were also reported by Xiao et al. (2016), who supplemented dairy calves with a culture of *S. cerevisiae*. The culture of *S. cerevisiae* alters the ruminal microbiota, reduces the accumulation of lactic acid, stabilizes ruminal pH, and prevents histopathological changes in ruminal papillae (Xiao et al., 2016; Shen et al., 2019). Knoblock et al. (2019) found no influence of the culture of *S. cerevisiae* in the expression of genes related to the cell junction of ruminal papillae in dairy cows during the transition period. Thus, the question remains as to whether this supplementation protects the ruminal papilla only by selecting the ruminal microbiota, or whether it could also stimulate greater tissue healing.

The autolyzed class of *S. cerevisiae* has higher levels of  $\beta$ -glucans and MOS than the culture class; thus, it mainly acts on the intestine. MOS have a high affinity for mannose-sensitive fimbrias type 1 found

in enterobacteria, such as *Salmonella* spp. and *Escherichia coli*. This mechanism of action decreases the ability of pathogenic microorganisms to adhere to the intestinal mucosa (Shurson, 2018). This promoted improvements in animal health and performance.  $\beta$ -glucans in contact with the intestinal mucosa alter the biological response by acting on the immune system and stimulating the activity of phagocytic cells in the systemic circulation (Rice et al., 2005; Shurson, 2018).

Thus, the blood phagocytes in the AUT group showed a greater effectiveness than those in the CON group, probably by the action of  $\beta$ -glucans or better absorptive intestine capacity, as this treatment promotes higher villus heights in the duodenum and jejunum, and higher villus:crypt ratios in the duodenum than in the other groups. During this treatment, autolyzed *S. cerevisiae* also acted on the rumen. It attenuated SARA to a lesser degree than the culture of *S. cerevisiae* as we observed lower ruminal inflammation scores and slight changes in ruminal papillary morphology. Autolyzed *S. cerevisiae* did not alter the ruminal epithelial microbiota but increased the expression of genes related to the cellular metabolism of the ruminal epithelium (Petri et al., 2020). It promotes an increase in the MI of these cells, which increases the cell replication capacity and tissue repair (Diaz et al., 2018).

The villus:crypt ratio is a useful criterion for estimating the digestibility and absorptive capacity of the small intestine. Thus, autolyzed *S. cerevisiae* provided a healthier intestinal epithelium as it promoted greater intestinal cell proliferation and absorption capacity, which may have also contributed to increased immunity.

## Conclusion

We concluded that both groups of *S. cerevisiae* increased the health of the animals compared with the CON group, especially supplementation with a culture of *S. cerevisiae*, which promoted a reduced alteration of ruminal papillae, better immune response, and lower occurrence of BRD than other groups; thus, this class is especially recommended for animals that are fed energetic diets. The autolyzed class of *S. cerevisiae* showed a greater effect on the intestinal tissue than the other groups and benefitted ruminal inflammation to a lesser extent.

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