

# Effect of *Saccharomyces cerevisiae* byproducts on milk phagocyte function and milk production in mid-lactation cows

## Efeito dos subprodutos de *Saccharomyces cerevisiae* na função de fagócitos lácteos e na produção de leite de vacas no meio do estágio da lactação

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### Highlights

The two byproducts of *S. cerevisiae* have different effects.

Supplemented cows are better adapted to stress factors such as thermal stress.

Supplementation with byproducts of *S. cerevisiae* minimizes the risks of mastitis.

### Abstract

*Saccharomyces cerevisiae* is a supplement option for ruminants due to its ability to stimulate the immune system and productivity; however, there are few studies that demonstrate the effectiveness of this yeast in dairy cattle, especially regarding its effect on milk phagocyte function. Thus, this study examined the effect of two presentations of autolyzed *S. cerevisiae* on milk phagocyte function and milk production in healthy Holstein cows from the third to the fifth months of lactation with somatic cell count (SCC) less than 200,000 cells mL<sup>-1</sup>. Ten animals received cell wall-rich *S. cerevisiae* autolysate (WC 15 g animal day<sup>-1</sup>); 8 received the cytoplasm-rich extract (CYT 5 g animal day<sup>-1</sup>) and 7 received a diet without supplementation (C, control) for 60 days. Weekly oxidative metabolism analysis of milk leukocytes, production and milk constituents was carried out. The oxidative metabolism of milk leukocytes was higher in the WC group than in the C group between D32 and D48 ( $P \leq 0.05$ ) and in the CYT group than in the C group between D24 and D40 ( $P \leq 0.05$ ). The production and percentage of milk fat increased in CYT at D48 and D56. It is concluded that the CYT group had a greater effect on productivity, while on immunity the effect was intermediate, compared to the WC group, which was efficient in improving the immunity of the mammary gland.

**Key words:** Immunity. Leukocytes. Milk. Oxidative metabolism. Yeast.

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## Resumo

*Saccharomyces cerevisiae* é uma opção de suplemento para ruminantes devido à sua capacidade de estimular o sistema imunológico e a produtividade; entretanto, ainda há poucos estudos que demonstrem a real eficácia das diferentes apresentações desta levedura em bovinos leiteiros, principalmente quanto ao seu efeito sobre a função dos fagócitos lácteos. Assim, o efeito de duas apresentações de *S. cerevisiae* autolisado na função fagocitária do leite e produção de leite de vacas da raça Holandes saudáveis entre o terceiro e quinto mês de lactação, com CCS inferior a 200.000 células mL<sup>-1</sup>. Para tanto, 10 animais receberam autolisado de *S. cerevisiae* ricos em parede celular (WC - 15 g animal dia<sup>-1</sup>); oito receberam o extrato rico em citoplasma (CYT - 5 g animal dia<sup>-1</sup>) e 7 receberam dieta sem suplementação (C - controle) por 60 dias. Análises do metabolismo oxidativo de leucócitos lácteos, produção de leite e constituintes do leite foram realizadas semanalmente. O metabolismo oxidativo dos leucócitos lácteos foi maior no grupo WC do que no grupo C entre D32 e D48 ( $P \leq 0,05$ ) e no grupo CYT do que no grupo C entre D24 e D40 ( $P \leq 0,05$ ). A produção de leite e a porcentagem de gordura do leite aumentaram no citoplasma (CYT) em D48 e D56. Concluiu-se que o grupo CYT teve um efeito maior na produtividade enquanto que na imunidade o efeito foi intermediário, comparando ao grupo WC que se mostrou eficiente na melhora da imunidade da glândula mamária.

**Palavras-chave:** Imunidade. Leucócitos. Leite. Metabolismo oxidativo. Levedura.

## Introduction

Bovine mastitis is a constant challenge in dairy farming, resulting in economic losses linked to loss of production, cost of medicines and milk disposal, in addition to its effects on animal and human health, either due to the presence of pathogenic microorganisms or the residue of antibiotics in the milk; these issues motivate the search for ways to prevent the disease by investing in measures that maximize the immunity of the animal (Langoni et al., 2017; Nocek et al., 2011).

The use of yeasts may be one such measure. In addition to improving nutrient absorption, yeasts can improve the immune status of the animal. *Saccharomyces cerevisiae* is the most widely used yeast in ruminants and is marketed in several presentations, either whole or autolyzed (Broadway et al., 2015).

While the intact form acts mainly in the rumen, the autolyzed form acts in the rumen and intestine. Its processing allows it to be separated into a soluble fraction composed of cytoplasmic content, which is rich in nucleotides responsible for modulating the immune response and aiding in the development of intestinal cells, in addition to offering more nutrients to the animal (Sauer et al., 2011). The insoluble fraction is rich in the cell wall, which has high proportions of  $\beta$ -glucans and mannan oligosaccharides responsible for stimulating the immunity of animals and selecting beneficial bacteria in the intestinal microbiota that increase nutrient absorption (Broadway et al., 2015).

The use of *S. cerevisiae* has already been studied in dairy cows, but the lack of details about its presentation and the very different experimental groups among the different studies do not allow comparison

of the results. While most studies found that different presentations of *S. cerevisiae* increased milk production and milk fat in cows at different stages of lactation (Aung et al., 2019; Nasiri et al., 2019; Zaworski et al., 2014), studies on immunity are scarcer and focus mainly on somatic cell count (SCC) and mastitis index. Adili et al. (2020) and Nocek et al. (2011) found that hydrolyzed *S. cerevisiae* promoted lower rates of mastitis in dairy cows in early lactation, but Aung et al. (2019) and Gimenes et al. (2020) did not find an influence on the SCC of cows at different stages of lactation.

Thus, both the presentation of *S. cerevisiae* and the animal model interfere with the response to the supplement. Heifers in the transition period, with higher SCC, have the most challenged immunity (De Vlieghe et al., 2012). Cows with more energetic diets and greater production potential have more digestive challenges (Valde et al., 2007). Thus, it is necessary to study the real benefit of each presentation of *S. cerevisiae* in each type of challenge.

Thus, this study aimed to evaluate the influence of two presentations containing autolyzed *S. cerevisiae* on the function of dairy phagocytes and on the production of healthy dairy cows in the middle of the lactation stage.

## Materials and Methods

This experiment was subjected to evaluation by the ethics committee for the use of animals of UNICENTRO and approved according to protocol 022/2020.

The experiment was conducted on a dairy cattle farm located in the municipality of Jordão, 50 km from Guarapuava, Paraná, in the south-central region of the state of Paraná, with geographical coordinates of 25° 23' 26" South latitude and 51° 27' 15" West Greenwich. The region has an altitude of approximately 1100 m and a moderate subtropical climate, with annual rainfall of 1.944 mm, minimum and maximum temperatures of 12.7 °C to 23.5 °C, and relative humidity of 77.9%. The experiment was conducted in the summer, with temperatures between 29°C and 14 °C and rainfall of 5.18 mm.

The property contained 70 lactating cows mixed in a *Compost Barn* system, with an average production of 20 liters of milk per day and a SCC of 300,00 cells mL<sup>-1</sup> of milk. The animals were fed three times a day, with the total mixed rations (TMR) in a feeding shed in an open field; with the aid of a unified mixer, each animal received 46 kg of TMR with a dry matter base composed of 60% corn silage, 18% commercial concentrate (Leite Max Avant 20T GP, Cooperativa AGRÁRIA, Entre Rios, PR, Brazil), 18% soybean meal; 3% predried oats (Table 1) and 1% mineral core (Bovigold®, DSM) (Figure 1).

Table 1

**Analysis of the chemical composition of the feed used in the total mixed ration (TMR) of the animals, based on dry matter**

Parameter	Corn silage (60%)	Concentrate (18%)	Soybean meal (18%)	Oat predried (3%)
Mineral matter % DM	3.49	8	6.84	6.5
Crude protein % DM	7.98	20	49.72	9.2
Ether extract % DM	3.57	4.8	4.51	5
Neutral detergent fiber % DM	35.01	20.82	9.28	64.2
Acid detergent fiber % DM	21.03	12	7.7	38.9
TDN % DM	76.39	84	81.18	60.61

DM= dry matter; MM= mineral matter; CP= crude protein; EE= ether extract; NDF= neutral detergent fiber; ADF= acid detergent fiber; TDN= total digestible nutrients.

Parameter	Mineral supplement Bovigold <sup>®</sup> , DSM
Calcium (Ca)	231,40 g/kg
Phosphorus (P)	40,00 g/kg
Sulphur (S)	20,00 g/kg
Magnesium (Mg)	25,00 g/kg
Potassium (K)	10 g/kg
Sodium Chloride (NaCl)	70 g/kg
Cobalt (Co)	15,00 mg/kg
Copper (Cu)	750,00 mg/kg
Chromium (Cr)	20 mg/kg
Iodine (I)	40,00 mg/kg
Manganese (Mn)	2.000,00 mg/kg
Selenium (Se)	22,00 mg/kg
Zinc (Zn)	2.850,00 mg/kg
Vitamin A	350.000,00 UI/kg
Vitamin D3	100.000,00 UI/kg
Vitamin E	2.000,00 UI/kg

Source: DSM, 2023.

**Figure 1.** Composition of the mineral supplement supplied to the animals in the total mixed ration (TMR) of all groups of the experiment.

The experimental design was completely randomized, conducted by a study model blinded to the treatment, where each animal was a sampling unit. For this purpose, 25 healthy multiparous Holstein cows were selected in the middle of the lactation stage (three to four months of lactation), with an average production of 31 liters of milk per day, obtained in two mechanical milkings following daily premilking; dipping and post dipping, using a mug with a dark bottom to detect clinical mastitis, was performed during all milkings. The California Mastitis Test (CMT) was performed every 15 days to detect subclinical mastitis. The animals were aged between four and six years old, with a body weight of 500 to 600 kg and body condition score (BCS) of three to four out of one to five, and a SCC below 200,000 cells mL<sup>-1</sup> of milk, which is the parameter used to detect the absence of subclinical mastitis. The animals were divided into three homogeneous groups according to milk production volume and

age at treatment: the WC group's (n = 10) diet was supplemented with *S. cerevisiae* autolyzed with higher cell wall concentrations (RumenYeast®, ICC, São Paulo, Brazil) at a dose of 15 g per animal per day<sup>-1</sup>; the CYT group (n=8) received a diet supplemented with *S. cerevisiae* strains rich in cytoplasmic content (Maxidigest®, ICC, São Paulo, Brazil) at a dose of 5 g per animal per day<sup>-1</sup>; and the control group (n=7) received no supplement.

The WC group received *S. cerevisiae* metabolites rich in amino acids and vitamins, mannan oligosaccharides and β-glucans. The CYT group received a concentrate of cytoplasmic content (extract) with a higher concentration of nucleotides and cobalamin (Figure 2). Supplementation took place right after the animals left the ordering station and they were arrested for feeding. Supplements were added to 1 kg of TMR once a day in the morning, and after food intake was observed, the remainder of the diet was fed and the cows released.

Parameter	CYT: MaxiDigest®	WC: RumenYeast®
Crude fiber	50 g/kg	90 g/kg
Crude protein	400 g/kg	350 g/kg
Mannan oligosaccharides	100 g/kg	120 g/kg
β-glucans	140	210 g/kg
Soluble metabolites	500 g/kg	300 g/kg
Free nucleotides	80 g/kg	-
Selenium	-	001 ppm
Thiamine	3,64 mg/kg	3,20 mg/kg
Cobalamin	558 mg/kg	2,79 mg/kg

Source: ICC Brazil (2021).

**Figure 2.** Ingredient composition of the two different supplements based on *S. cerevisiae* yeast tested in this experiment: WC = Yeast rich in cell wall and CYT = Yeast rich in cytoplasm.

Samples were collected at weekly intervals for 60 days: D0, D8, D16, D24, D32, D40, D48 and D56. The animals were subjected to milk collection and physical examination with evaluation of heart and respiratory rates, ruminal movements and rectal temperature, in addition to the staining of the mucosa and lymph nodes. Approximately 40 mL of milk was collected in bottles containing the preservative Bronopol, and 100 mL of milk was collected in Falcon bottles without preservative. The milk was obtained directly from the closed system of the milking machine, representing a sample of the entire morning milking of the four teats of each cow. In addition, the dark-bottomed mug test was performed twice a day to detect the onset of clinical mastitis, and total milk production was measured at weekly intervals.

In the milk samples, the 40-mL aliquot was sent to the accredited laboratory (APCBRH/PARLPR, Curitiba, Paraná, Brazil) for analysis of SCC, lactose, fat and protein by FTIR and flow cytometry methods.

The 100 mL aliquot of milk without preservative was subjected to two centrifugations ( $1000 \times g$  for 15 min at  $4^\circ\text{C}$ ) for separation of the cell *pellet*. The supernatant and fat were discarded, and the *pellet* was resuspended in 1 mL of phosphate-buffered saline (PBS). This suspension was then subjected to a cell count and viability test using the Trypan blue technique. All samples were adjusted to  $1 \times 10^6$  viable cells  $\text{mL}^{-1}$  for cellular metabolism and cytological evaluation.

To evaluate cellular metabolism, we incubated 100  $\mu\text{L}$  of the lacteal cell suspension ( $1 \times 10^6$  viable cells  $\text{mL}^{-1}$ ) with equal parts of 1% NBT solution (Sigma®, São

Paulo, Brazil) and stimulated the sample with 5  $\mu\text{L}$  of 12 phorbo myristate 13 acetate ( $300 \text{ ng mL}^{-1}$  PMA, Sigma®, São Paulo, Brazil) for 30 min at  $37^\circ\text{C}$ . The reaction was stopped by the addition of 2000  $\mu\text{L}$  of ice-cold EDTA (3 mM), and then the extracellular NBT was removed by washing with PBS and centrifugation ( $400 \times g$  for 8 min at  $4^\circ\text{C}$ ). The supernatant was then discarded, and the cell *pellet* was fixed to the bottom of the tube with methanol (Synth®, São Paulo, Brazil). The cells were then dissolved in KOH (Synth®, São Paulo, Brazil) (3 M, 120  $\mu\text{L}$ ) and DMSO (Dimesol®, MarcoLab, São Paulo, Brazil) (99%, 140  $\mu\text{L}$ ), and the suspension was read spectrophotometrically at 630 nm (Thermo Plate®, São Paulo, Brazil) in duplicate, with intervariability confidence of less than 5%.

For cytological evaluation, 100  $\mu\text{L}$  of the lacteous cell suspension ( $1 \times 10^6$  viable cells  $\text{mL}^{-1}$ ) was cytocentrifuged ( $400 \times g$  for 6 min). The cell *pellet* was fixed with methanol (Synth®) and stained with Panótico Rápido®. For the differential count of cells, a light microscope was used, and 300 cells were counted at 1000x magnification and classified into macrophages, neutrophils and lymphocytes.

The data collected for each variable were subjected to analysis of variance with comparison of the means at 5% significance using the statistical software InStat GraphPad. The data were evaluated by comparing the means of the time-treatment interaction using ANOVA. When statistical significance was detected, the means of each group were compared by the interaction time using Tukey's posttest, and the means of each moment by the interaction treatment using Tukey's test. For the SCC variables, the

data were transformed into logs of 10 and evaluated by the tests described above. Data with a significance level lower than or equal to 5% ( $P \leq 0.05$ ) were considered significant.

## Results and Discussion

The experiment started with 10 cows in each experimental group. These animals did not show alterations in the dark background examination or in the CMT. However, since the first collection, 3 animals from the control group and 2 from the CYT group had SCC greater than 200,000 cells  $\text{mL}^{-1}$  in milk, during the entire experiment, and therefore they were removed from the experiment.

None of the animals showed changes on physical examination or in the examination of milk on a dark background. All groups had subclinical mastitis on D40 (SCC: greater than 200,000 cells  $\text{mL}^{-1}$  in milk), which altered the cellular profile by increasing neutrophils and reducing lactic macrophages, at which time there was a failure in milking machine vacuum, a problem that was resolved in the following days.

The SCC, the percentages of lactate macrophages and neutrophils and the oxidative metabolism of lactic phagocytes were influenced by treatment, time and the time x treatment interaction (Table 2) and are detailed in Figure 3.

**Table 2**

**Evaluation of SCC, differential count and oxidative metabolism of milky leukocytes from cows treated or not with the yeast *S. cerevisiae***

	WC n: 10	CYT n:8	Control n:7	SD	Time X treatment	Time	Treatment
SCC Log10	4.62ab	4.52a	4.76b	0.51	0.01	0.02	0.04
Macrophages %	87.2a	84.9b	84.9b	3.28	0.02	0.008	0.01
Neutrophils %	4.5a	5.1ab	5.5b	2.30	0.02	0.009	0.004
Lymphocytes %	4.7a	6.1a	4.7a	2.67	Ns	Ns	Ns
OM (od)	0.073a	0.072ab	0.069b	0.001	0.02	0.01	0.03

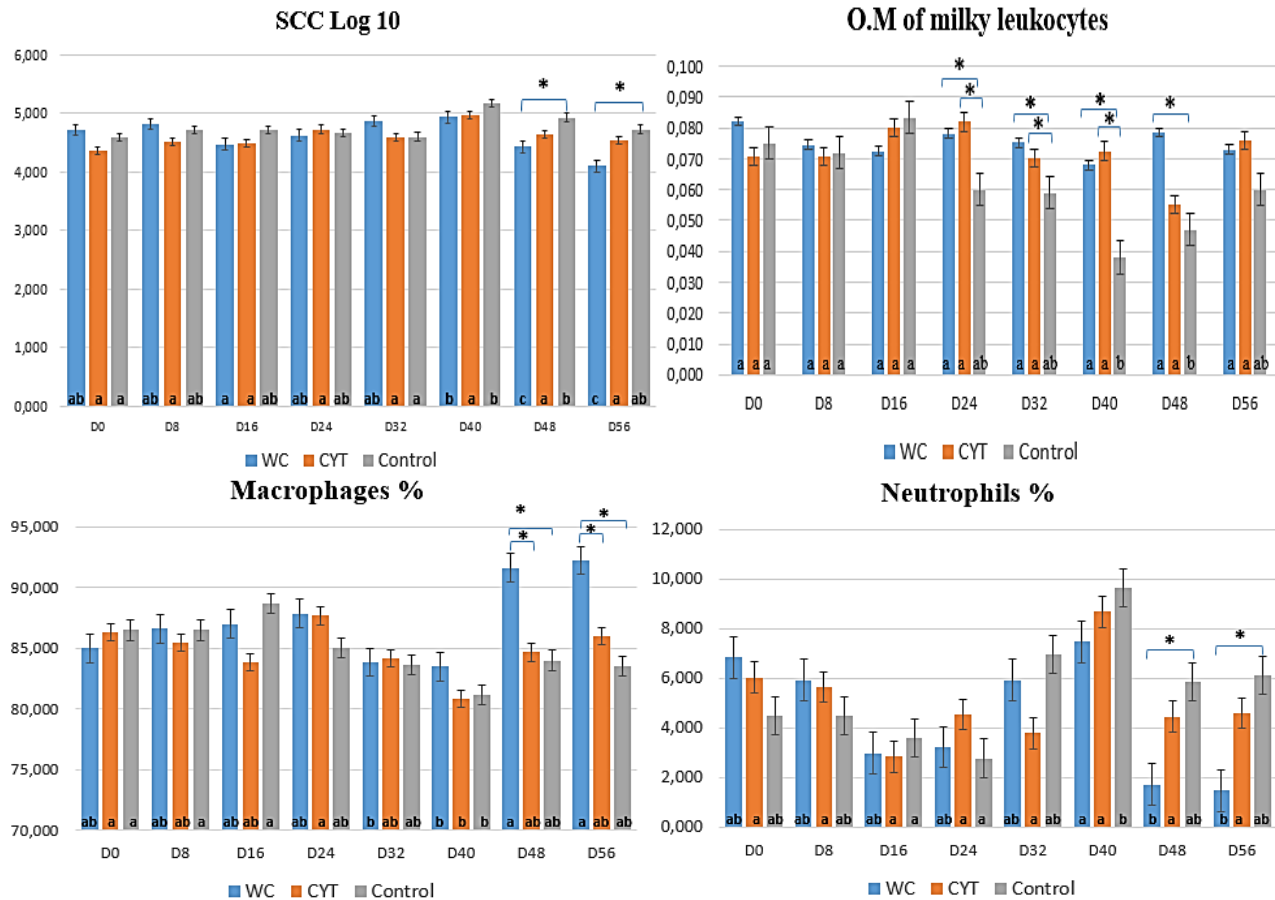
WC: yeast with cell wall; CYT: cytoplasm-rich yeast; Control: control; SCC: somatic cell count; OM: leukocyte oxidative metabolism; OM: optical density; Ns: not significant. Data are expressed as the mean and standard deviation (SD). a, b: Different letters indicate significant differences ( $P \leq 0.05$ ).

In the WC group, one animal had subclinical mastitis on D32, and two had subclinical mastitis on D40. On D40, there was an increase in the SCC, which declined on D48 and D56 and was lower than on D0, D8, D32 and D40 ( $P=0.001$ ). Additionally, on D40, the percentage of macrophages was

lower than on D48 and D56 ( $P=0.009$ ), and the percentage of neutrophils was higher ( $P=0.009$  and  $P=0.009$ ). Regarding treatment, on D48 and D56, SCC was lower than in the Control group ( $P=0.05$  and  $P=0.04$ ), while the percentage of macrophages was higher and the percentage of neutrophils lower than that

of the other groups ( $P= 0.001$  and  $P=0.009$  macrophages and  $P=0.004$  and  $P=0.001$  neutrophils) (Figure 3). Additionally, between D32 and D48, the oxidative metabolism of

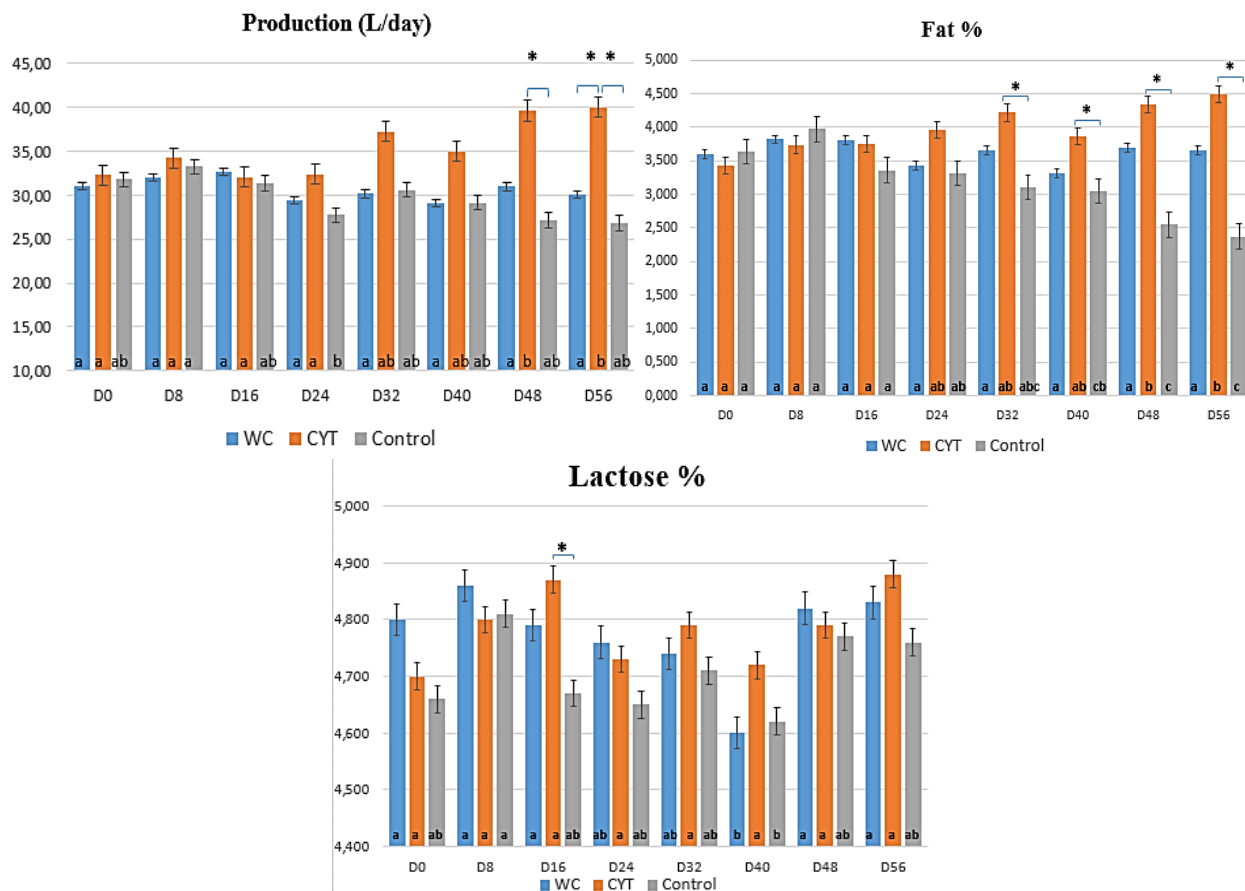
the dairy cells was higher than that of Control group, with no time interaction ( $P=0.05$ ;  $P=0.04$  and  $P=0.03$ ) (Figure 4).



**Figure 3.** SCC, differential count and oxidative metabolism of milky leukocytes from cows treated or not with the yeast *S. cerevisiae*.

WC: yeast with cell wall; CYT: cytoplasm-rich yeast; Control: control; Data are expressed as the mean and error bar. \*Indicates difference between treatments at the time of study ( $P\leq 0.05$ ); a, b, c: Different letters indicate significant difference between time points within the same group ( $P\leq 0.05$ ).





**Figure 4.** Volume and constituents of milk produced from cows treated or not with the yeast *S. cerevisiae*.

WC: yeast with cell wall; CYT: cytoplasm-rich yeast; Control: control; Data are expressed as the mean and error bar. \* Indicates difference between treatments at the time of study ( $P \leq 0.05$ ); a, b, c: Different letters indicate significant difference between time points within the same group ( $P \leq 0.05$ ).

In the CYT group, one animal presented subclinical mastitis on D40, without an increase in SCC. At this time, there was a reduction in macrophages compared to D0 and D24 ( $P=0.05$ ) without a significant increase in neutrophils. The oxidative metabolism of dairy cells was higher than in the Control group between D24 and D40 ( $P=0.05$ ;  $P=0.05$ ;  $P=0.04$ ), with no time interaction (Figure 3).

In the Control group, one animal presented subclinical mastitis on D40 and another on D40 and D48. There was an increase in SCC at these times compared to D0 and D32 ( $P=0.03$ ). On D40, there was a decrease in lactic acid macrophages compared to D0, D8 and D16 ( $P=0.01$ ) and an increase in neutrophils compared to D16 and D24 ( $P=0.01$ ). For this group, there was a reduction in lactic leukocyte oxidative metabolism on D40 and D48 compared to the first few days ( $P=0.001$ ).

Milk production and the percentages of milk fat and lactose were affected by the treatment, time and the time x treatment interaction (Table 3) and are detailed in Figure 2. At D24, a reduction in milk production was observed in group C compared to the other time points (P=0.01). At this time, an environmental temperature of 29 °C with low relative humidity was recorded, and climatic conditions were milder in previous and subsequent moments. While milk production and milk fat levels remained stable throughout the time points for the

WC group, both increased in the CYT group on D48 and D56 compared to the earliest days (P=0.01 and P=0.05), when production was also higher than that in the other groups (P=0.05 and P=0.03). The milk fat content was higher than that of the Control group from D32 onward (P=0.05, 0.05; 0.003 and 0.002). The Control group showed a reduction in fat from D40 onward (P=0.03) and a reduction in the percentage of lactose on D40 compared to D8 (P=0.05) (Figure 4). Dairy proteins were not affected by treatment or treatment time.

**Table 3**  
**Milk yield and constituents of cows treated or not with the yeast *S. cerevisiae***

	WC n: 10	CYT n:8	Control n:7	SD	Treatment time	Time	Treatment
Production (L Day <sup>-1</sup> )	30.44ab	35.66a	29.14a	8.29	0.01	0.001	0.03
Fat %	3.64ab	3.95a	3.30b	1.60	0.01	0.009	0.04
Lactose %	4.77a	4.78a	4.26b	1.30	0.05	0.001	0.04
Protein %	3.0	3.36	3.12	0.33	Ns	Ns	Ns

WC: yeast with cell wall; CYT: cytoplasm-rich yeast; Control: control; L: liters; Ns: not significant. Data are expressed as the mean and standard deviation (SD). a, b: Different letters indicate significant differences (P≤0.05).

It was observed that autolyzed *S. cerevisiae* rich in the cell wall (WC) promoted greater immune growth because it stimulated the oxidative metabolism of milk phagocytes for a longer time and reduced the SCC, especially at the end of the experiment when the milky cell profile was composed of a higher percentage of macrophages. Concentrations of cytoplasm (CYT) promoted an increase in milk and fat production in the final phase of the evaluation and presented an intermediate immune increase because there was an increase in temporary leukocyte oxidative metabolism, which did not interfere with the SCC or the percentage of lactic

macrophages. It is noteworthy that these effects were more pronounced at times of greater challenge to the animals, such as thermal stress on D24 and failure of the milking machine vacuum on D40, because there was a decrease in production and a higher incidence of subclinical mastitis in the Control group, which did not occur in the treated groups.

The increase in the frequency of subclinical mastitis and SCC in all groups on D40 is attributed, but with statistical significance in the WC and CYT groups, to the milking machine vacuum system

failure, which promoted postmilking residual milk in the mammary gland, responsible for an inflammatory process of the udder that predisposed the cows to bacterial colonization, promoted neutrophilic influx to the mammary gland and increased SCC (Sordillo, 2018).

Later, the Control group remained high in SCC, while there was a reduction in the WC group and stabilization in the CYT group. This can be explained by the influence of *S. cerevisiae* autolysates, which are more active in situations of greater challenge. Previous studies found that supplementation with *S. cerevisiae* reduced the SCC of dairy cows in the postpartum period (Nocek et al., 2011; Zaworski et al., 2014; Yuan et al., 2015) but did not influence the SCC in dairy cows in the middle of the lactation stage (Dehghan-Banadaky et al., 2013).

In this study, the cows were not under a higher challenge, as they were pluriparous cows and in the middle of the lactation stage, therefore, without a negative energy balance, which would affect energy support for the animal's immune cells and might favor immunosuppression. In addition, the animals had low SCC (below 200,000 cells mL<sup>-1</sup>), indicating good management and a little challenged immune system that ensured the health of the mammary gland (Gonçalves et al., 2018). At the moment of greatest challenge, represented by D40, the increase in residual milk diluted the immunological factors of the animals, requiring greater activation of phagocytes to combat infectious agents (Sordillo, 2018). Which in group Control took longer to be reestablished, while in the treated groups occurred more quickly, when the SCC levels returned to normal in the following week.

This can be confirmed by the stability of the oxidative metabolism of lactic leukocytes in the treated groups and the decrease in the Control group between D40 and D48 in the time interaction. In the WC group, the maintaining of the oxidative metabolism of lactic leukocytes allowed macrophages, the main resident phagocytes of the mammary gland, to eliminate the pathogens that invaded the tissue, without the need to recruit neutrophils from the blood to the mammary gland from D48 onwards, the which resulted in a decrease in SCC at D48 and D56. In the CYT group, this function was less effective, as shown by the higher percentages of neutrophils at D48 and D56 and maintenance of SCC, indicating that for this mammary gland to remain healthy, it was necessary to flood the tissue with neutrophils, since macrophages alone failed to inhibit the pathogens (Sordillo, 2018).

The mannans and  $\beta$ -glucans of the cell wall of *S. cerevisiae* act as immunomodulators by increasing phagocytosis and cytokine production by macrophages and activating neutrophils and T and B lymphocytes (Jensen et al., 2008). Although supplementation with this yeast presentation also improved lymphocyte function in previous studies, there were no changes in the percentage of milky lymphocytes throughout the study, cells that correspond to approximately 1 to 2% of milky cellularity (Sordillo, 2018). The cytoplasm-rich extract of *S. cerevisiae* contains nucleotides that activate the replication of immune cells, which, despite interfering very little in the function of phagocytes, may have contributed to a greater number of them, which promotes a more efficient immune response (Ibrahim & El-Sayed, 2016).

Oxidative metabolism is the production of reactive oxygen species (ROS) by phagocyte enzymes leading to the destruction of phagocytosed pathogens, and its decreased function indicates lower phagocyte competence (Tizard, 2014). Thus, the incubation of milk cells with the nitroblue toltrazuril reagent produces the formation of a bluish compound inside the phagocytes called formazan, the intensity of intracellular production of reactive oxygen species by phagocytes such as neutrophils and macrophages, and the greater or lesser production. The concentration of ROS changes the staining intensity of the compound, thus predicting the ability of phagocytes to destroy pathogens through oxidative metabolism and is considered an indirect *in vitro* microbicidal test (Takeshima et al., 2021). Although the increase in phagocyte efficiency by *S. cerevisiae* wall-based supplements has already been observed in blood leukocytes of heifers, it had not yet been tested in dairy phagocytes (Ryman et al., 2013).

Regarding dairy production, there was a decrease in milk production in all groups at D24, with statistical significance only in the Control group. This was associated with thermal stress, where the temperature reached 29 °C with low relative humidity. During this period, it was observed that the supplemented groups better adapted to this stressor. Similar results were found in studies using live yeast and yeast walls during the summer with high temperatures, when the treated groups better adapted to these conditions and maintained consumption, production and immunity (Brandão et al., 2016; Perdomo et al., 2020).

Subsequently, the animals of the CYT group showed increased milk production and fat content on D48 and D56 both in the treatment and time interaction. In the WC group, there was stability in milk production and milk fat percentage, and for the CYT group, there was a decline in production on D40 and D48 and a reduction in the percentage of fat from D40 and D56 and in the percentage of lactose in D40.

The cytoplasmic content of the autolyzed *S. cerevisiae* extract provides substrate for ruminal microorganisms, favoring better diet utilization and feed conversion ratio, resulting in increased milk and fat production due to increased propionate production (Poppy et al., 2012). In addition to yeast, the CYT group's diet had a considerable percentage of cobalamin (precursor of vitamin B12), which in ruminants corresponds to the metabolism of propionate and gluconeogenesis, essential for the energy and glucose requirements to aid in milk production (Graulet et al., 2007).

This explains the increase in milk and fat production from the sixth week for the CYT group. Similar results were observed in studies with live and hydrolyzed yeasts and yeast culture, which increased milk production and fat content in the milk of the treated groups; while the yeast presentations differed from ours, the preparations also provided nutrients to the ruminal microorganisms, but in different percentages to the supplements used in our study, making each yeast presentation unique and with different mechanisms of action and composition according to the manufacturing technology of each (Nasiri et al., 2019; Zaworski et al., 2014).

The reduction in fat, lactose and volume of milk produced in group Control can be explained by the occurrence of inflammation of the mammary gland, in which the pathogens consume fat and lactose present in milk, in addition to promoting destruction of the glandular tissue (Malek dos Reis et al., 2013). As this inflammation was attenuated in the WC group because the immune cells were more competent in the *in vitro* test, the constituents remained unchanged throughout the experiment.

Lactose levels usually show a decrease in animals affected by mastitis due to the presence of pathogens that ferment lactose, which would explain its decrease in D40 in the WC and CYT groups (Feitosa, 2014). For the CYT group, there was a point increase in the percentage of lactose on D16 and D40, which we could not justify. The milk protein levels were not affected by the treatments, similar to the findings when using dry yeast in cows in early lactation and  $\beta$ -glucan in the diet of lactating cows (Gimenes et al., 2020; Li et al., 2021).

Thus, the use of autolyzed *S. cerevisiae* can be used in the diet of dairy cows to minimize the incidence of mastitis and increase milk production, even in a situation of low challenge during milk production.

With this in mind, it is speculated that *S. cerevisiae* autolyzed in more challenged herds may bring more expressive results. It is believed that WC supplementation will bring a greater immune increase in herds with higher SCC and in animals in the transition period, a situation in which the immune system is more challenged and the organism is more prone to immunosuppression. Supplementation with CYT will result in better results and higher productivity in herds with less challenge, due

to its average performance in stimulating immunity.

## Conclusion

Supplementation with autolyzed *S. cerevisiae* from the WC group was more efficient in stimulating innate immunity, reducing SCC and maintaining mammary gland health. The extract of *S. cerevisiae* from the CYT group caused an increase in milk production, both in volume and in components such as fat, and promoted an intermediate increase in the immune response of the mammary gland than the WC group.

## Acknowledgments

This study was supported by ICC Brazil and the Coordination for the Improvement of Higher Education Personnel (CAPES), n.001.

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