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Fresh yeast additives improve immune parameters and reduce respiratory disease in heifers finished in feedlots

Aditivos de levedura viva melhoram parâmetros imunes e reduzem doenças respiratórias em novilhos terminados em confinamento

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Highlights:
Fresh yeast additive improves neutrophilic function heifers finished in feedlots.
Fresh yeast additive minimizes inflammatory status of heifers finished in feedlots.
Fresh yeast additive minimize Respiratory disease of heifers finished in feedlots.

Abstract

Although cattle feedlot presents productive advantages, this management generates stress, which may impact immunity and increase the incidence of respiratory diseases. Because the immunostimulatory potential of yeasts, this study aimed to verify whether supplementation of cattle with fresh yeast promotes an increase in innate immunity and, consequently, reduces the occurrence of respiratory diseases in heifers finished in feedlots. A total of 32 heifers, finished in feedlots, were randomly divided into two treatments and 16 repetitions: A control group (n=16; 7 g day $^{-1}$ per animal of corn kernel) and a yeast group (n=16, 7 g day $^{-1}$ per animal of the commercial product with fresh yeast additive). At day 0, 16 days after acclimatisation to the feedlot, and at days 28, 56 and 84, leukogram, serum haptoglobin, oxidative metabolism neutrophil, and indicators of respiratory diseases (nasal temperatures, nasal secretion score, and histopathological examination of lung) were evaluated. The yeast group had lower blood neutrophil counts (P = 0.02), higher neutrophil oxidative metabolism (P = 0.04) than the control group after 56 days of confinement. There was a lower frequency of animals from the yeast group with purulent nasal secretion on days 28 and 84 (P = 0.0001 and 0.008) and with histopathological lesion of pneumonia at slaughter day (P = 0.0001). The yeast group also presented lower nasal temperatures than the control group on days 28 and 84 (P = 0.02 and P= 0.08). Thus, fresh yeast additives attenuated the effects of the feedlot system in the heifer immune system and contributed to a reduction of respiratory diseases.

Key words: *Saccharomyces cerevisiae.* Oxidative metabolism. Pneumonia. Bovine.

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Resumo

Embora confinamento de bovinos apresente vantagens produtivas, este manejo gera estresse, podendo impactar a imunidade e aumentar a incidência de doenças respiratórias. Tendo em vista o potencial imunoestimulante das leveduras, este trabalho objetivou verificar se a suplementação de bovinos com levedura fresca promove incremento da imunidade inata e consequentemente, diminui a ocorrência de afecções respiratórias de novilhas terminados em confinamento. Para tanto 32 novilhas terminadas em confinamento, foram divididas aleatoriamente em dois tratamentos, com 16 repetições cada, formando o grupo controle (n = 16; 7 g dia $^{-1}$ por animal de caroço de milho) e o grupo levedura (n = 16, 7 g dia $^{-1}$ por animal do produto comercial com aditivo de levedura fresca). No dia 0, 16 dias após a aclimatação ao confinamento, e nos dias 28, 56 e 84, avaliou-se o leucograma, haptoglobina sérica, metabolismo oxidativo de leucócitos e indicadores de doenças respiratórias (temperatura nasal, escore de secreção nasal e exame histopatológico do pulmão). O grupo levedura apresentou menor contagem de neutrófilos no sangue (P = 0.02), maior metabolismo oxidativo dos neutrófilos (P = 0.02) e menores níveis séricos de haptoglobina (P = 0.04) do que o grupo controle após 56 dias de confinamento. Houve menor frequência de animais do grupo levedura com secreção nasal purulenta nos dias 28 e 84 (P = 0.0001 e 0,008) e com lesão histopatológica de pneumonia no dia do abate (P = 0.0001). O grupo levedura também apresentou temperaturas nasais mais baixas do que o grupo controle nos dias 28 e 84 (P = 0.02 e P = 0.08). Assim conclui-se que o aditivo de levedura fresca atenuou os efeitos do sistema de confinamento no sistema imunológico de novilhas e contribuíram para a redução de doenças respiratórias, o que traz impactos importantes na pecuária seja por reduzir custos com tratamento, evitar resistência bacteriana a antibióticos, como diminuir a morbidade de doenças respiratórias entre os animais .

Palavras-chave: S*accharomyces cerevisiae.* Metabolismo oxidativo. Pneumonia. Bovinos.

Introduction

Although there are advantages to finishing cattle in feedlots, mainly related to cost reduction and better carcass finishing, the system may favour the occurrence of diseases, particularly infectious diseases, such as Bovine Respiratory Disease (BRD) (Cusack, Mcmeniman, & Lean, 2003; Edwards, 2010). BRD is responsible for up to 44.1% of morbidity in feedlots, generating an economic impact of US $11.85 million per year (Cusack et al., 2003; Malafaia, Granato, & Costa, 2016).

This high incidence is associated with stress that results from several factors, such as the animals' entrance into the feedlot, transport, agglomeration of the animals in restricted spaces, and abrupt diet change. This can promote an increased secretion of cortisol, which may be responsible for the reduction of immune system efficiency (Enemark, 2008; Torquist & Rigas, 2010). In addition, high energy diets, containing 50% concentrate or more, are commonly used in the feedlot. These diets cause multiple episodes of rumen acidosis and inflame the organ (Cusack et al., 2003). This inflammatory cascade promotes secretion of proinflammatory cytokines by tissue macrophages that immunomodulate the inflammation, promoting an increase of acute phase proteins, a reduction of circulating neutrophils due to their marginalisation to the rumen, and a reduction of phagocytic activities, which seems to contribute to the reduction of general immune activity and increase the occurrence of infectious diseases (Torquist & Rigas, 2010; Sato, 2015).

In order to avoid possible ruminal disorders, feed additives are frequently used. Among the different products on the market, yeasts, especially *Saccharomyces cerevisiae*, are promising natural additives. Yeasts can be used as a fresh or lyophilised live formulation or in the form of yeast-based products (Broadway, Carroll, & Sanchez, 2015). Although it is understood that both types of formulation increase the immune response by

direct or indirect action there still contradictory results regarding the effects of live *Saccharomyces cerevisiae yeast* on the immune system and health of cattle, but apparently higher concentrations of yeasts show greater immunogenic gain (Broadway et al., 2015).

While Keyser, McMeniman, Smith, McDonald and Galyean et al. (2017) found that live yeast did not interfere with BDR morbidity in feedlot beef cattle, Finck et al. (2014) found that live yeast not only reduced BDR morbidity in feedlot cattle, but also increased their response to treatment against this disease.

In the above-mentioned research, lyophilised yeast was used in different dosages and concentrations. Lyophilisation reduces the viability of yeast cells per gram of product, reducing their colonisation of the gastrointestinal tract by causing morphological changes in yeast cell membranes, which results in decreased concentrations of *Saccharomyces cerevisiae* in the ruminal environment (Callaway & Martin, 1997). Therefore, we believed that the use of fresh yeast will allow a higher concentration of viable yeast to reach the rumen than lyophilised yeast and, consequently, minimise the impacts of confinement on the bovine immune system more efficiently, thus reducing the occurrence of respiratory diseases in feedlot cattle. This research aimed to evaluate the effects of a fresh yeast additive on the innate immunity and health of the respiratory tract of heifers finished in feedlots.

Materials and Methods

This experiment was approved by the committee on ethics in animal use, CEUA-UNICENTRO-002/2017.

Animals and sample collection

The experimental design was randomised and developed as a blind model study. Thirty-two crossbred ½ Angus-Nelore heifers (body weight 317±70 kg and 11.9±4 months) were used. These animals were from a property near the experimental unit and were housed in feedlot pens at the Núcleo de Produção Animal (NUPRAN) at the Universidade Estadual do Centro-Oeste (UNICENTRO), located in Guarapuava, Paraná, Brazil. The animals were homogeneous distributed considering the animals' weight and body condition score between the control group (C, n=16), which was fed 7 g day $^{-1}$ corn kernel per animal, and the treatment group (Yeast, n=16), which 7 g day $^{-1}$corn kernel per animal plus a live yeast additive: 10^7 UCF g^{-1} of *Saccharomyces cerevisiae* ATCC9080 in a high humidity organic matrix (SILEV PLUS, Sachar Feeds, Cascavel, Paraná, Brazil). Prior to the experiment, the animals were acclimatised to the experimental facilities and the new diet. Their previous diet (100% corn silage) was replaced by a diet of 50% commercial concentrate (Cooperativa Agrária, Guarapuava, Paraná, Brazil) and 50% silage corn. The concentrate was gradually incorporated over sixteen days, until reaching the proportion of 50%. The animals were fed ad libitum twice daily at 06h00 and 17h30. To standardise the volume of feed provided, the feed left in the trough was weighed daily and adjusted so that 5% of the dry matter remained. After adjustment, the corn kernel or corn kernel plus yeast was added according to the groups. Sample moments took place on days 0, 28, 56 and 84 (table 1). At 105 days the animals were slaughtered in a commercial slaughterhouse.

Table 1
Chemical composition of feed used in animal feeding and average values of experimental ration, based on total dry matter

Parameter	Corn silage	Concentrate	Experimental ration*
Dry matter, %	33.83	90.40	62.12
Mineral matter, % DM	2.51	6.36	4.44
Crude protein, % DM	8.44	20.20	14.32
Ethereal extract, % in DM	2.65	2.05	2.35
Neutral detergent fiber, % DM	46.14	31.47	38.80
Acid detergent fiber, % DM	25.98	13.08	19.53
Lignin, % DM	8.43	4.73	6.58
Total digestible nutrients, %	68.66	78.68	74.17
Ca, %	0.14	1.67	0.91
P, %	0.22	0.58	0.40

*[1] Premix guarantee level per kg of concentrate: vit. A: 16000 IU; vit D3: 2000 IU; vit. E: 25 IU; S: 0.36 g; Mg: 0.74 g; Na: 3.6 g; Co: 0.52 mg; Cu: 22.01 mg; F: 18.00 mg; I: 1.07 mg; Mn: 72.80 mg; Se: 0.64 mg; and Zn: 95.20 mg.

Sample analysis

To verify the innate immunity heifers, blood samples were collected on days 0, 28, 56 and 84 by venopuncture of the external jugular in a glass vacuum tube containing heparin (8 mL) for evaluation of leukocytes oxidative metabolism, in a glass vacuum tube containing ethylene diamine tetraacetic acid (EDTA) (4 mL) for quantification of leukocytes, and in a glass vacuum tube without anticoagulant for serum haptoglobin measurement (8 mL). To evaluate the heifers' oxidative metabolism, the blood tubes were refrigerated until processing. No more than three hours elapsed between collection and processing. The colorimetric reduction technique of nitroblue tetrazolium (NBT) was used, according to Choi, Kim, Cha and Kim (2006) with modifications. Briefly, 100 µL of whole blood was incubated with 5% NBT solution (Sigma®) and stimulated with 5 µL of phorbol 12-myristate-13-acetate (PMA, 300 ng / mL, Sigma®) for 30 minutes at 37 °C. Subsequently, the reaction was stopped by the addition of 2000 µL of ice-cold EDTA (3 mM), the red cells were disrupted by osmotic lysis, the outer NBT was removed after washing with methanol (100%, Synth®, São Paulo, Brazil), and the cells were dissolved with KOH (3 M, 120 µL, Synth®, São Paulo, Brazil) and DMSO (99%, 140 µL, Dimesol®-MarcoLab,São Paulo, Brazil). Next, the suspension was read in 620 nm spectrophotometry at optical density (OD). Samples were taken in duplicate. The mean of the results was recorded for each animal, with an intra-variability index of less than 5%.

The leukogram was measured in a Neubauer chamber and cell population counts based on morphometric characteristics were performed on blood smears. For haptoglobin dosages, whole blood was centrifuged at 3500 rpm X 15 minutes at room temperature, and blood serum was frozen at -20 °C until processing. The samples were analysed in duplicate in a commercial set of bovine haptoglobin by the ELISA technique (Finetest, Wuhan Fine Biotech LTDA, Wuhan, China).

Indicators of respiratory disease were evaluated on day 0, 28, 56 and 74 by observing nasal secretion and nasal temperature. These observations were done at the same time of day, on each of five consecutive days after each experimental timepoint.

The occurrence of lung lesions was evaluated on the day of slaughter. The animals' nasal temperatures were measured with an infrared thermometer (AKSO, AK30 New, China) directed at the nasal cavities of the bovines, at approximately 20 cm, with the observer outside the animals' pens. The bovine nasal secretion score was evaluated by direct inspection, and assigned as either one (unilateral or bilateral mucosal secretion) or two (bilateral purulent secretion). After each five day observation period, the lowest nasal temperatures and the median nasal secretion scores were calculated for each sampling timepoint. The frequency of animals with each nasal secretion score was then calculated. On the day of slaughter, fragments, of approximately 2 cm^2, of ventral cranial lobe were collected in the transition area between normal tissue and consolidation areas. The tissues were then fixed in 10% formaldehyde for 48 hours, embedded in paraffin, sectioned for hematoxylin and eosin (HE) stained histopathological slides and, finally, observed via transmitted light microscopy. Pneumonia scores were assigned according to the degree of the severity of circulatory and degenerative changes. A score one was assigned to animals with no visible lesions or mild hyperaemia of the cranioventral lobes without any consolidation. A score two was assigned to animals with consolidation of up to 50% of the cranioventral lobes. A score three was assigned to animals with consolidation of 51 to 100% of the cranioventral lobes based on recorded lung lesions (Ceribasi, Ozkaraca, Ceribasi, & Ozer, 2014) and the frequency of animals with each pneumonia score was calculated.

Statistical methods

The data collected were evaluated using Instat Graphpad statistical software (GraphPad Software, La Jolla, CA, USA). For the evaluation of oxidative metabolism and nasal temperature, the analysis of variance (ANOVA) for repeated samples and Tukey test were performed. The parametric t-test was performed to compare each moment of the different groups. The frequency analyses of nasal secretion and lung injury scores were performed using the chi-square test. The variables haptoglobin serum and blood leukocytes were non-normal data (Kolmogorov and Smirnov test) and, therefore, a non-parametric test was performed, and Dunn's post-test was performed for comparison within each timepoint. To compare the treatment interaction, a non-parametric t-test was performed. Differences were considered significant at $p \leq 0.05$, and a trend to significance was defined as $0.05 < P < 0.10$.

Results

The evaluations of neutrophil oxidative metabolism and serum haptoglobin are shown in Table 2. Analysis of time interactions indicate that C had increased serum haptoglobin, with day 56 and 84 statistically higher than day 0 and 28 ($P = 0.001$), whereas Yeast only presented a statistically significant increase of serum haptoglobin on day 84 ($P = 0.001$). For treatment interaction, haptoglobin serum was higher on day 0 for Yeast compared to C ($P = 0.04$), and higher in C than Yeast on days 56 and 84 ($P = 0.04$ and 0.09, respectively).

Table 2
Phagocytes oxidative metabolism and serum haptoglobin of heifers finished in feedlots with live yeasts included in the diet

Variable	Groups		D0	D28	D56	D84	p^X	Reference value[1]
Oxidative metabolism O.D.	Control n=16	Least	0.57abA	0.46aA	0.74bA	0.88bA	0.0001	--
		SEM	0.06	0.03	0.06	0.02		
	Yeast n=16	Least	0.70aA	0.44bA	0.93aB	0.84aA	0.0001	
		SEM	0.10	0.03	0.05	0.02		
	p^y		0.27	0.65	0.02	0.24		
Serum haptoglobin (mg dL^{-1})	Control n=16	Median	10.8aB	94.7abA	111.5bB	189.9bA	0.0011	0- 35.00
		CI	6.30-20.9	11.5-250.4	40.6-236.4	133.5-251.9		
	Yeast n=16	Median	31.78aA	58.4aA	29.3aA	149.6bA	0.0011	
		CI	7.9-60.64	32.67-49.62	2.32-77.78	87.0-217.9		
	p^y		0.04	0.3	0.04	0.09		

1. Tothova, Nagy and Kovac (2014).
CI- confidence interval, SEM: mean standard error, O.D. optical density. px- interaction time, Tukey test (oxidative metabolism) and Dunn test (serum haptoglobin), Different lower case letters in the same row indicate different statistical difference between moments and py- interaction treatment, T Test, different upper case letters in the same column indicate statistical difference between groups, $p < 0.05$.

Both groups presented a statistically significant reduction in neutrophil oxidative metabolism in comparison to the other timepoints (P = 0.0001) on day 28. Subsequently, this function increased at day 56 in both groups, being of greater magnitude in Yeast than C (P = 0.02).

The values of the leukocytes are contained in table 3. We can verify that Yeast had higher counts of total leukocytes and neutrophils at day 0 in comparison to C (P = 0.09 and P= 0.01, respectively). Although most of the animals had a normal leukocyte count or a count that was slightly increased by lymphocytosis and monocytosis since the first collection, it was possible to observe greater discrepancies between the groups at day 56, when the animals from C had lower absolute counts of leukocytes, higher neutrophil counts and higher neutrophil lymphocyte ratios (N/L) compared to Yeast (P = 0.09, P = 0.02, P = 0.003 respectively).

Table 3
Leukogram of heifers finished in feedlots with live yeasts included in the diet

Variables	Groups		D0	D28	D56	D84	pX	Reference value[1] x1000 mm^3 ul^{-1}
Leukocyte x1000 mm^3 ul^{-1}	Control n=16	Median	11.80aA	14.15abA	12.67abA	15.125bA	0.003	4.0 - 12.0
		CI	10.3-13.67	12.07-15.08	11.76-14.5	13.96-15.8		
	Yeast n=16	Median	13.10aA	12.60aA	14.10aA	14.80aA	0.24	
		CI	12.08-15.8	11.71-15.5	13.17-16.05	13.9-16.3		
		py	0.09	0.48	0.09	0.91		
Neutrophil x1000 mm^3 ul^{-1}	Control n=16	Median	2.4aA	3.45bA	3.45bA	3.42bA	0.03	0.6 - 4.0
		CI	2.03-4.4.	2.90-4.08	2.93-4.21	2.80-3.84		
	Yeast n=16	Median	3.32aB	2.95aA	2.68aB	2.61aA	0.14	
		CI	3.32-3.98	2.45-3.79	2.22-2.97	1.91-3.34		
		py	0.01	0.14	0.02	0.07		
Eosinophil x1000 mm^3 ul^{-1}	Control n=16	Median	0.19aA	0.14aA	0.30aA	0.31aA	0.16	0 - 2.4
		CI	0.154-0.40	0.10-0.56	0.17-0.57	0.20-0.60		
	Yeast n=16	Median	0.27aA	0.27aA	0.50aA	0.63aA	0.24	
		CI	0.24-0.66	0.26-0.80	0.24-0.59	0.39-1.03		
		py	0.24	0.08	0.51	0.12		
Basophil x1000 mm^3 ul^{-1}	Control n=16	Median	0aA	0aA	0aA	0aA	0.373	0 - 0.2
		CI	0.0-0.04	0.01-0.11	0.01-0.08	0.00-0.05		
	Yeast n=16	Median	0aA	0aA	0aA	0aA	0.09	
		CI	0.02-0.12	0.02-0.15	0.01-0.09	0.02-0.06		
		py	0.14	0.74	0.99	0.58		
Lymphocyte x1000 mm^3 ul^{-1}	Control n=16	Median	7.28aA	9.81abA	8.25abA	10.79bA	<0.001	2.5 - 7.5
		CI	6.30-8.11	7.90-10.34	7.55-10.07	9.85-11.8		
	Yeast n=16	Median	7.38aA	8.40abA	9.88bA	9.86abA	0.002	
		CI	6.40-9.23	7.43-9.97	8.80-11.3	9.37-11.9		
		py	0.65	0.46	0.14	0.44		
Monocyte x1000 mm^3 ul^{-1}	Control n=16	Median	1.33aA	0.66bA	0.69abA	0.50bA	<0.001	0.02 - 0.84
		CI	0.98-1.51	0.54-0.89	0.50-0.82	0.41-0.69		
	Yeast n=16	Median	0.98aA	0.60abA	0.63abA	0.56abA	0.02	4.0 - 12.0
		CI	0.79-1.40	0.60-1.49	0.63-1.18	0.49-1.10		
		py	0.21	0.95	0.74	0.92		
Neutrophil Lymphocyte Ration	Control n=16	Median	0.40abA	0.37abA	0.41bA	0.27aA	0.04	0.3- 0.5
		CI	0,30-0,60	0,31-0,53	0,33-0,52	0,25-0,38		
	Yeast n=16	Median	0.50aA	0.30abA	0.25bB	0.23bA	0.0008	
		CI	0.35-0.64	0.23-0.47	0.19-0.31	0.17-0.35		
		py	0.40	0.28	0.003	0.33		

1. Tornquist and Rigas (2010).
CI- confidence interval,pP^x- interaction time, Dunn test, Different lower case letters in the same row indicate different statistical difference between moments and py- interaction treatment, T Test, different upper case letters in the same column indicate statistical difference between groups, p <0.05.

C presented leukocytosis by neutrophilia and lymphocytosis on day 28 in relation to the initial timepoint (P = 0.003, P = 0.03, P = 0.001 respectively), whereas, in Yeast, the leukocyte and neutrophil counts tended to be stable. A lymphocyte increase was only observed on day 56 in relation to day 0 (P = 0.002).

For the signs of respiratory diseases, there was a progressive, statistically significant increase d in nostril temperatures between day 0 and day 56 for C (day 0 different from day 28 and day 56 P = 0.001). In Yeast, these increases occurred only at days 56 and 84 in relation to the initial moments (P = 0.0001). The C had a higher nostril temperatures and higher frequency of purulent nasal secretion than Yeast at days 28 (27.30ºC x 25.75ºC P = 0.02 and 38.89% x 11.11% P= 0.0001) and 84 (30.37ºC x 29.62ºC P = 0.08 and 55.55% x 27.78 % P= 0.008). At slaughter day, no animals presented score three pneumonia, but the frequency of score two pneumonia in C was statistically higher than Yeast (61.11% x 16.66%; P = 0.0001).

Discussion

We observed that fresh yeast attenuated the inflammation and the immune system depression that the feedlot system causes in animals. This was illustrated by the fact that heifers treated with a fresh yeast additive exhibited fewer indications of respiratory diseases than heifers that were not treated.

Haptoglobin is an acute phase protein that is considered an early indicator of inflammation (Tothova et al., 2014). In healthy cattle, haptoglobin levels do not exceed 35 mg dL^{-1} (Hanthorn et al., 2014). Serum haptoglobin levels exceeded the normal range on days 28, 56, and 84 in both groups. Numerically, levels were consistently higher in C than in Yeast, but the difference was only statistically significant on day 56. This increase indicates a greater inflammation in C than Yeast. Haptoglobin immunomodulates inflammation by activating or depressing the functions of blood neutrophils (Hanthorn et al., 2014; Tothova et al., 2014). Thus, it is believed that on day 28 of the experiment some ruminal damage occurred and that this damage caused a mild and non-statistically significant increase of serum haptoglobin in both groups. This acute inflammation could immunomodulated the leukocytes functions and reduced the activity of phagocytes oxidative metabolism at this time.

Leukocytes oxidative metabolism is the respiratory activity of phagocytes, mediated for active enzyme complexes, which produce reactive oxygen species responsible for the destruction of internalised pathogens (Burgos, Conejeros, Hidalgo, Werling, & Hermosill, 2011). Thus, the fact that both groups showed a reduction in this activity at 28 days indicates that the blood neutrophils of the animals of both groups were phagocytosing and eliminating fewer circulating pathogens, thus making the animals more susceptible to infections, particularly bacterial infections, which are the most dependent on neutrophilic functions (Burgos et al., 2001). Similarly, Reck (2017) also observed that feedlot steers receiving high energy diets had a reduced phagocytosis and a neutrophil stimulated oxidative metabolism.

Inflammation was still present at days 56 and 84 in C, but it was only seen on day 84 in Yeast. This type of prolonged inflammation could culminate in a chronic increase of the serum haptoglobin levels. Possibly this chronic inflammation did not interfere to a great extent in the phagocyte's oxidative metabolism. A similar result was found by Reck (2017) who studied feedlot-finished cattle that were fed a high energy diet.

The lower absolute counts of leukocytes, higher neutrophil count, and higher neutrophil lymphocyte ratio in C compared to Yeast, specially at day 56, reinforce the conclusion that C presented a higher inflammatory status, probably due to ruminal lesions caused by a high energy diet. This ruminal damage promoted increase of leukocytes, neutrophils, and

lymphocytes in the blood circulation, as observed by Ceroni, Turmalaj, Lika and Duro (2012), when studying cattle fed high energy diets. In Yeast, the probiotic attenuated the inflammation status because Yeast stabilised the ruminal pH (Broadway et al., 2015). So, as neutrophil activity was increased at this time, the animals did not require changes in the circulating leukocytes, a fact also observed by Finck et al. (2014). They observed that live yeast supplementation decreased the neutrophil lymphocyte ratio of LPS-challenged cattle.

In our previous research, we verified that this same fresh yeast additive improved dry matter digestibility in bovine fed a high energy diet, justified by the maintenance of ruminal health and prevention of injuries in the digestive tract of animals given live yeast additives (Stadler et al., 2019). Thus, the current finding supports the hypothesis that fresh yeast additives can reduce the inflammation status of heifers finished in feedlots.

There were higher counts of total leukocytes, neutrophils, and serum haptoglobin at day 0 in Yeast than C. Although the levels of leukocytes and serum haptoglobin were within the reference values for the species (Torquist & Rigas, 2010), no explanation was found for the neutrophilia observed at day 0. It is possible that these animals presented infections, although no animal showed a characteristic sign of disease, such as decreased food intake, apathy, decubitus, or respiratory manifestations, at this time. Another explanation could be the stress of transport, a new environment, or change in diet, but the animals of C experienced the same situations and did not show elevated leukogram counts or serum haptoglobin levels.

We verified that the improvement of these immune parameters had contributed to the health of the respiratory tract of the animals. C showed a higher nostril temperature, frequency of purulent nasal secretion and frequency of pneumonia than did Yeast. According to Baptista et al. (2017), the main infectious disease of feedlot cattle is BRD, with up to 44% morbidity. Thompson, Stone and Schultheiss (2006) found pneumonia lesions in 43% of feedlot cattle at the time of slaughter.

Schaefer et al. (2007), Timsit, Bareille, Seegers, Lehebel and Assié (2011), consider the presence of purulent nasal secretions and increased face temperature measured by infrared thermometer to be non-invasive measures and early markers of respiratory disease. So, the higher nasal temperatures and greater number of animals with purulent nasal secretion in C demonstrated that this group had impaired respiratory tract health, which was confirmed by the higher frequency of animals with pneumonic lesions at slaughter day.

Respiratory disease indicators occurred with greater intensity on days 28 and 84 in C. At day 28, both groups had a reduction in neutrophil oxidative metabolism and C had numerically higher levels of serum haptoglobin. As live yeasts favour a nonspecific phagocytic activity of alveolar macrophages, which are the primary resident lung cells responsible for local defence (Cross, 2002). It is reasonable to conclude that the treatment contributed to Yeast group animals exhibiting milder clinical signs of respiratory diseases when compared to C.

At day 56, C continued to present a reduced leukocyte oxidative metabolism and higher levels of serum haptoglobin and circulating neutrophils compared to Yeast, indicating that C animals presented an inflammatory status with a higher infection response than Yeast, although the signs of respiratory disease were similar in both groups. This increased inflammatory status continued on day 84, when C continued to present higher serum haptoglobin levels and circulating neutrophils, as well as more indicators of respiratory diseases than Yeast.

We cannot explain whether the higher inflammation found in C was triggered by ruminal injuries, respiratory disease, or both. There is still disagreement in the literature regarding the

mechanisms of action of live yeasts. While some authors favour the idea that the growth of cellulolytic bacteria and stabilisation of ruminal pH attenuates ruminal stress (Sato, 2015), others suggest that the yeast acts directly on the immune system, improving the efficiency of phagocytes, although these mechanisms remain unknown (Cross, 2002).

However, the results of this research were more significant than those of Keyser et al. (2007), Finck et al. (2014) in which live lyophilised yeast did not decrease or only numerically decreased the BRD morbidity in feedlot cattle. This may have occurred because of different management of the animals between the studies. In the previous studies, there was a higher climatic amplitude and there were more animals per pen. Therefore, the animals were exposed to a greater competition for animal dominance, which could cause more BRD than was seen in our study (Baptista et al., 2017). In addition, there were differences in the form of live yeast used, which can affect the concentration of viable yeast that reaches the rumen. Further studies are necessary to compare lyophilised yeast with fresh yeast with the same concentrations of *Saccharomyces cerevisiae* to determine the form that is most efficient at improving the immune systems of feedlot cattle.

Conclusions

Fresh yeast attenuated the inflammatory effects and the depression of innate immune response in feedlot-finished heifers as demonstrated by inducing lower serum haptoglobin levels and neutrophil counts and higher phagocytes' oxidative metabolism. Fresh yeast also contributed to the health of the respiratory tract in feedlot-finished heifers, as demonstrated by lower indicators of respiratory disease (purulent nasal secretion and nasal temperature) and fewer instances of pneumonia in treated animals at the time of slaughter.

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Conflict of interest statement

The authors have no competing interests.

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