SEMINA
Ciências Agrárias

DOI: 10.5433/1679-0359.2024v45n2p509

Influence of autolyzed *yeast* on the health of immunologically immature calves

Influência da levedura autolisada na saúde de bezerras imunologicamente imaturas

Katlin Zamarchi Primels[1]; Ana Carolina Araujo Abreu[2]; Bianca Milla[2]; Sarah Naiverth Oliveira[2]; Karoline Vintureli Felicio[3]; Meire Christina Seki[4]; Heloisa Godoi Bertagnon[5]*

Highlights

Autolyzed yeast improves weight gain of calves.
Autolyzed yeast attenuates the side effects of the respiratory vaccine.

Abstract

Autolyzed yeast is a supplement option for calves due to its ability to stimulate the immune system and performance; however, there are few studies about the effect of different yeast derivatives. Autolyzed yeast is rich in immunomodulatory substances, such as mannan oligosaccharides and β-glucans. These substances enhance the innate and humoral immunity of calves, resulting in greater intestinal and respiratory health. Thus, the objective of the study was to evaluate whether supplementation with autolyzed yeast improves the health of calves, in the challenge of naturally acquired Eimeriosis and bovine respiratory disease (BRD). Twenty Holstein calves aged 15 days, which had already suckled colostrum, were studied for 36 days. At 15 days of life all animals had naturally acquired Eimeriosis. They were challenged with an intranasal BRD vaccine at 30 days of age. The Supplemented group (n=10) received autolyzed yeast (10 g animal -1 day -1), once a day in milk for 36 days, and the Control group (n=10) was not supplemented throughout the experiment. Blood neutrophil: lymphocyte ratio, oxidative metabolism of neutrophils (OM), serum immunoglobulins and haptoglobin, occurrence of diarrhea, BRD, and weight gain were measured. After vaccination, all animals increased the blood neutrophil:

[1] Student of the Master's Course of the Postgraduate Program in Veterinary Sciences, Universidade Estadual do Centro Oeste, UNICENTRO, Guarapuava, PR, Brazil. E-mail: katlinprimel@gmail.com
[2] Students of the Undergraduate Course in Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail: anacarolinaaabreu@gmail.com; biancamilla36@gmail.com; naiverths@gmail.com
[3] Student of the Veterinary Medicine Improvement Course, UNICENTRO, Guarapuava, PR, Brazil. E-mail: karoline011@hotmail.com
[4] Profª Drª of the Undergraduate Course in Veterinary Medicine, UNICENTRO, Guarapuava, PR, Brazil. E-mail: meireseki@hotmail.com
[5] Profª Drª of the Master's Course of the Postgraduate Program in Veterinary Sciences, UNICENTRO, Guarapuava, PR, Brazil. E-mail: hbertagnon@hotmail.com
* Author for correspondence

Received: Aug. 11, 2023 Approved: Apr. 08, 2024

lymphocyte ratio, but only the control group showed a 20% reduction in OM three days after the vaccine. The supplement affected the immune response, preserving OM, increasing serum IgA levels by 10% (P=0.006), and reducing haptoglobin serum levels (P=0.05). In addition, the supplement attenuated diarrhea and BRD, which allowed for greater weekly weight gain (difference of 2 kg between treatments, P<0.05), concluding that the autolyzed yeast showed some evidence that it can increase the health of calves challenged with Eimeriosis and the BRD vaccine.
Key words: Bovine Respiratory Disease. Eimeriosis. Immunity. Reactive oxygen species. *Saccharomyces cerivisiae.*

Resumo

Saccharomyces cerevisiae é uma opção de suplemento para bezerros devido a capacidade de estimular o sistema imune e a performance dos animais, no entanto há poucos estudos os subprodutos derivados desta levedura. A *S cerevisiae* concentra substâncias imunomoduladoras como mananoligossacarídeos e β-glucanos, que estimulam a imunidade inata e humoral de bezerras, o que refletiria em maior saúde intestinal e respiratória. Desta maneira o objetivo do estudo foi avaliar se a suplementação com *S cerevisiae* autolisada melhora a saúde de bezerras, no desafio sanitário de Eimeriose naturalmente adquirida e doença respiratória bovina (DRB). Foram estudadas 20 bezerras da raça Holandesa com 15 de vida, com colostragem adequada, durante 36 dias. Todas apresentavam Eimeriose naturalmente adquirida aos 15 dias de vida, e foram desafiados com vacina intranasal para doença respiratória bovina aos 30 dias de vida. O grupo Suplementado (n=10) recebeu levedura autolisada (10 g animal^{-1}), uma vez ao dia, durante 36 dias no leite e o grupo Controle (n=10) não foi suplementado. Razão neutrófilo/linfócito, metabolismo oxidativo de neutrófilos (MO), imunoglobulinas séricas e haptoglobina, bem como ocorrência de diarreia, DRB e ganho de peso foram medidos. Após a vacinação, todos os animais aumentaram a relação neutrófilo: linfócito, mas apenas o grupo controle apresentou redução de 20% na MO três dias após a vacina. O suplemento afetou a resposta imune, preservando MO, aumentando os toeres séricos de IgA em 10% (P=0.006) e reduzindo os teores séricos de haptoglobina (P=0.05) Além disso, o suplemento atenuou a diarreia e a DRB, o que permitiu um ganho de peso médio semanal de 2 kg a mais que o C (P<0,05), concluindo que há evidências de que a *levedura* autolisada aumenta a saúde de bezerras desafiadas com Eimeriose e com a vacina contra DRB.
Palavras-chave: Doença Respiratória Bovina. Eimeriose. Espécies reativas de oxigênio. Imunidade. Saccharomyces cerevisiae.

Introduction

The health of cattle is important for the maintenance and improvement of national productivity, motivating research on the main diseases from the beginning of life of these animals. Diarrhea and pneumonia are major causes of death in calves up to 1 year of age (Atkinson et al., 2017). The multiple etiologies of diarrhea complicate the causal diagnosis, but diarrhea caused by *Eimeria* sp., one of the etiological agents frequently found in the feces of calves, is common (Lopez-Osorio et al., 2020). Furthermore, pneumonia comprises a condition called bovine respiratory disease (BRD) involving the triad of stress, viruses,

and bacterial diseases. This condition has a high incidence, particularly in dairy calves between 30 and 60 days of age, a period in which passive immunity begins to decrease and active immunity is not mature (McGill & Saco, 2020).

Several protocols have been prescribed to prevent and alleviate diarrhea and pneumonia in calves, especially to improve animal immunity and thus minimize the use of antibiotics, whose excessive use favors bacterial resistance. Autolyzed yeast cells are immunostimulants, which decrease the severity and incidence of diarrhea in neonatal calves regardless of etiology, either by stimulating cell–cell communication, increasing the efficiency of blood phagocytes, or increasing the production of intestinal IgA (Fomenky et al., 2018; Villot et al., 2020).

S. cerevisiae also decreases the severity and incidence of viral pneumonia in newborn calves (Mahmoud et al., 2020; McDonald et al., 2021). *S cerivisiae* e *Enterococcus faecalis* attenuated the inflammation of the respiratory tract produced by the vaccine against BRD viruses in growing calves and potentiated the humoral vaccine response (Bertagnon et al., 2024). Its immunostimulatory effects are linked to polysaccharide such as mannan oligosaccharides, present in *S cerevisiae,* responsible to stimulating cell–cell communication and phagocyte responses, and a polysaccharide β-glucans present in both microorganisms, responsible to compete for adhesion sites in the gastrointestinal tract which prevent the colonization of pathogenic bacteria in mucosal sites (Oelschlaeger, 2010; Uyeno et al., 2015).

This yeast is marketed in different presentations and the technology for obtaining it is essential to maintain its active concentration and thus establish its mode of action, and a stronger or weaker effect. It is believed that presentations with more β-glucans and mannan oligosaccharides have greater immunostimulating power than the intact cell presentation. One of the technologies to achieve this presentation is autolysis, which separates the yeast from its cell wall (Elghandour et al., 2022), but it is not known whether such a presentation promotes greater intestinal and respiratory health in immunological immature calves.

This study aims to determine whether autolyzed *yeast* improves the immunity of calves challenged with Eimeriosis and bovine respiratory viruses' vaccine, decreasing the occurrence of diarrhea and BRD.

Materials and Methods

This experiment was approved by the Ethics Committee -UNICENTRO- 025/2021.

Environment, farm management and animal selection

The study was conducted on a commercial property located in the Witmarsun region of Palmeira, Paraná, Brazil. A total of 20 Holstein calves aged 15 days and weighing 48.15 ± 5.4 kg, which had suckled colostrum, were selected to the conduction of this study. The animals were distributed into the supplemented (S) and control (C) groups, containing 10 animals each, according to the supplementation treatment they would be given.

The experiment was conducted between June and August. From birth, the calves remained in a shed separate from the other animal categories, in individual stalls (1m X 1.5 m), with straw and sand bedding and separated from the other stalls by a wooden slatted structure that still allowed communication between calves. The animals were fed warm, pasteurized whole milk (5% body weight at each feeding), in a bucket with nipples twice a day until the end of the experiment. The animals received water, hay, and commercial calf starter (CANCELA Animal nutrition for high-production calves, Cooperativa Witmarsum, Paraná, Brazil) ad libitum.

As for management, the farm separated the calves from the dam at birth and supplied colostrum at 10% of live weight in the first 2 hours of life. The dams and calves were not vaccinated against respiratory diseases or agents causing diarrhea. As inclusion criteria, only calves that were not persistently infected (PI) with bovine viral diarrhea virus (BVDV) (as evaluated with a BVDV antigen enzyme-linked immunosorbent assay -ELISA- kit, Idexx, São Paulo, Brazil) and that had >6.2 g dL-1 serum protein and >2400 mg dL^{-1} IgG in the first week of life.

For supplementation, 10 g of autolyzed yeast per animal per day was added to the milk just before feeding to make sure it was completely consumed. The autolyzed yeast used was an autolyzed *Saccharomyces cerevisiae* derived from ethanol sugarcane industry (Rumenyeast®, ICC Brazil) The dose utilized allowed an intake of 1,4 g of mannan oligosaccharides and 2,8 g of β-glucans. Control did not receive supplementation.

Eimeriosis

When the animals were 15 days old, all animals had *Eimeria* sp. in the feces diagnosed by the Willis–Mollay technique. Five animals from each group had normal stools and five had diarrheal stools. All the animals were treated with a single dose of oral toltrazuril (Baycox, São Paulo, Brazil; 15 mg kg^{-1}) and two doses of injectable trimethoprim sulfadoxine at a 15-day interval (Borgal, MSD, São Paulo, Brazil; 15 mg kg^{-1}) on day 0. When dehydration above 5% was detected, the calves received 2 L of oral fluid in the feeding bottle once a day, according to the management adopted by the farm (Hydrafeed, Kersia, Rio Grande do Sul, Brazil; 100 g).

Bovine respiratory disease challenge

At 30 days of age, the calves received 2 mL of the intranasal vaccine against BRD in a single dose (Inforce 3, Zoetis, São Paulo, Brazil), composed of a modified live virus (MLV: IBR, PI-3 and BRSV). One mL was applied to each nostril, and the calves' heads were kept elevated for approximately 20 seconds.

Parameters evaluated

The animals were evaluated daily during the 5 weeks for the occurrence of fever, apathy, presence of diarrhea and pneumonia, hyporexia, degree of hydration, and body weight. Diarrhea was assessed by fecal score, classified daily from 1 to 3 according to Ollivett et al. (2009). Fecal scores of 2-3 for at least 3 days were considered diarrhea, and

the duration of the condition was recorded, as well as the amount of recurrence of the disease. The data of fecal score was transformed into the medians of each group for each week of the experiment. Eimeriosis was checked for weekly using the Willis–Mollay and egg counting gram techniques.

The respiratory score was verified by Wisconsin and California scoring systems according to Decaris et al. (2022) with modifications, based on 3 criteria: nasal discharge or ocular discharge, rectal temperature and cough. For each parameter, the animal can receive a score of 0 to 3 according to the severity of the signs. One point is assigned to each variable, reaching a score between zero and nine. When the sum was greater than four, the calf was clinically evaluated to identify pneumonia. Pneumonia cases were treated with enrofloxacin (7.5 mg kg^{-1}, SC, Kinetomax, Bayer, São Paulo, Brazil).

When the calves were 15, 33, 36, and 51 days old, blood samples were collected to measure the oxidative metabolism of blood neutrophils, the neutrophil:lymphocyte ratio, and serum immunoglobulin IgG and IgA. Serum haptoglobin was measured when the calves were 15 and 33 days old.

Neutrophilic oxidative metabolism was assessed by the nitroblue tetrazolium (NBT) colorimetric test, and the neutrophil:lymphocyte ratio was analyzed in blood smears. For this purpose, 4 mL of blood was collected in vacuum tubes containing heparin. The samples were kept refrigerated for a maximum of 3 hours before processing. Then, 100 µL of blood was incubated with 100 µl of a 1.5% solution of NBT and 50 µl of PMA at 37 °C for 30 minutes. Blood smears were taken and stained with rapid Panoptic hematology stain, in which we counted the percentage of neutrophils in relation to lymphocytes and the positivity of the NBT reaction for every 100 neutrophils, as described by Ciarlini et al. (2002).

For the measurement of immunoglobulins and haptoglobin, 8 mL of blood was collected in vacuum tubes free of anticoagulants and allowed to sediment naturally for 12 hours at room temperature. The blood serum was aliquoted and frozen until the end of all collections. IgA and IgG and haptoglobin were measured by commercial ELISA kit (Bovine IgA ELISA or Bovine IgG ELISA, Biomatik, Toronto, Canada; Bovine haptoglobin, Finetest, Wuhan, China). The samples were diluted according to the manufacturer's instructions, with an intra-assay coefficient of variation of <8% and coefficient of variability <10% for immunoglobulins assays and they were 6.6% and 7.8%, respectively for haptoglobine. All samples were analyzed in duplicate.

Statistical analysis

For statistical analysis, the data were analyzed using the InStat statistical program (GraphPad). Significance was determined at a P value of ≤0.05, while a P value of ≤0.10 indicated a trend. Data regarding fecal score, serum haptoglobin, and neutrophil:lymphocyte ratio were nonparametric and analyzed using the Kruskal-Wallis test. In cases where an interaction between time and treatment was observed, the Mann-Whitney test was conducted to compare treatments, and the Dunn test was employed to compare different time points. Diarrhea and BRD frequencies were analyzed using the chi-squared test.

Other data passed normality (Kolmogorov-Smirnov) and variance (Bartlett) tests and were subjected to ANOVA. In instances where a time × treatment interaction was detected, Student's t test was used to compare treatments at each time point, and Tukey's test was utilized to compare different time points for each treatment.

The sample size calculation for the immunological parameters evaluated was based on the formula = 2 SD^2 (Zα/2 + Zβ)^2/d^2, Zα/2 = Z 0.05/2 = Z 0.025=1.96 (Z Table) for type 1 error at 5% Zβ = Z 0.20 = 0.842 (Z Table) at 80% statistical power and d = is the effect size = difference between the averages. As parameters for variable "d" (difference between means) we adopted a variation of 20% ("small" difference) and 50% ("large" difference).

Results and Discussion

During the experiment, all calves had at least one episode of diarrhea (fecal score 2-3, minimum duration of 3 consecutive days, and mean duration of 5 days), and one animal in each group had diarrhea lasting 36 days. In group S, none of the animals presented oocysts of *Eimeria* sp. in the third week, while three animals in group C still had them. In the 5th week, all animals were negative for *Eimeria* sp. although some animals still had diarrhea.

Diarrhea lasting and recurrence were higher in group C than in group S (P=0.03 and 0.02, respectively, table 1). The C group presented softer stools (higher fecal score) than the S group in the 2nd and 3rd weeks of the experiment (P=0.0002 and P=0.01, respectively, Figure 1).

Table 1
Recurrence and duration of diarrhea in calves supplemented (S) or not (C) with *Yeast*

Diarrhea recurrence (%)	None	1 recurrence	2 or more recorrence	P*
C (n=10)	40	40	20	0,02
S (n=10)	60	40	0	
Diarrhea lasting (%)	1 week	2 weeks	3 or more weeks	
C (n=10)	30	10	60	0,03
S (n=10)	20	50	30	

*chi-squared test.

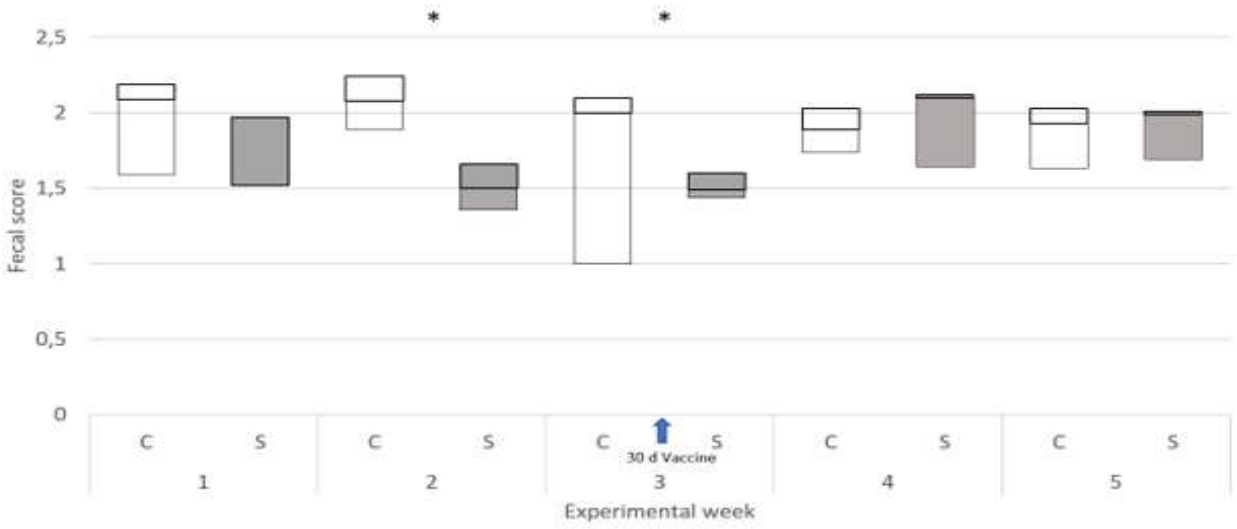

Figure 1. Weekly fecal score (median, 1st and 3rd quartiles) in calves supplemented (S) or not (C) with *Yeast.*
* Indicates difference between treatments (P ≤0.05, Dunn's test between times and Student's t test between treatments).

Autolyzed yeast supplementation enhanced the immune response of immunologically immature calves facing challenges of Eimeriosis and BRD. Additionally, these immunonutrients reduced the duration and recurrence of diarrhea while also diminishing the incidence of BRD in the studied animals. Thus, there is some evidence suggesting that this treatment positively impacted the health of the calves. However, further studies with larger sample sizes are warranted to corroborate these findings.

Although Eimeriosis is often asymptomatic and self-limiting, a high rate of bloody diarrhea was observed in animals, either because they were immunologically immature or because of the high contamination of the environment. The type of calf stall difficulted the environmental disinfection, and the physical contact between the animals allowed the diarrhea agent to remain in the environment and contaminated them (Lopez-Osorio et al., 2022; Thaler Neto et al., 2014). Treatment with toltrazuril and trimethoprim sulfadoxine is effective, but the presence of *Eimeria* sp. in the feces of some animals 15 days after treatment indicated the occurrence of reinfections, while diarrhea without the presence of oocysts in the feces indicates secondary bacterial infections that multiply in the intestinal villi that was destroyed by Eimeriosis (Lopez-Osorio et al., 2022).

Supplementation with the autolyzed yeast decreased the lasting and recurrence of diarrhea, as well as the fecal score of calves in the second and third weeks of the experiment. Our previous studies demonstrated that *S cerivisiae* supplementation promote an

immunomodulation around 15 days after supplementation in calves (Flores et al., 2019; Bertagnon et al., 2024). Despite this, Villot el al. (2020) found the supplementation with yeast in fermentative culture medium promoted an increase of IgA intestinal mucosa and interfered with the intestinal microbiome of neonatal calves, 7 days after the supplementation of health calves. Thaler Neto et al. (2014) found that yeast minimized the occurrence of diarrhea of different etiologies in neonatal calves. β-Glucans and mannan oligosaccharides favor the growth of beneficial microorganisms in the intestine, preventing pathogenic bacteria from causing opportunistic diarrhea. In addition, they increase the height of the villi and crypts, what increase the absorption of nutrients (Elghandour et al., 2022).

The frequency of animals with respiratory disease and pneumonia is shown in Figure 2. All animals had no clinical changes in the respiratory system in the first week, and only one calf in group C showed changes in the second week of the experiment (at 24 days of life), with fever, spontaneous productive cough, and crackles in the cranioventral lobes. One day after the intranasal vaccination, 90% of the animals in each group presented a positive cough reflex, bilateral mucopurulent nasal secretion, and crackles on tracheal auscultation, without fever A total of 40% (4/10) of group C and 10% (1/10) of group S evolved to fever (above 39.2° C) lasting 2-3 days, productive spontaneous cough, bilateral mucopurulent nasal secretion, and crackles in the lung lasting 10-15 days, so they were treated on the first day that pneumonia was identified.

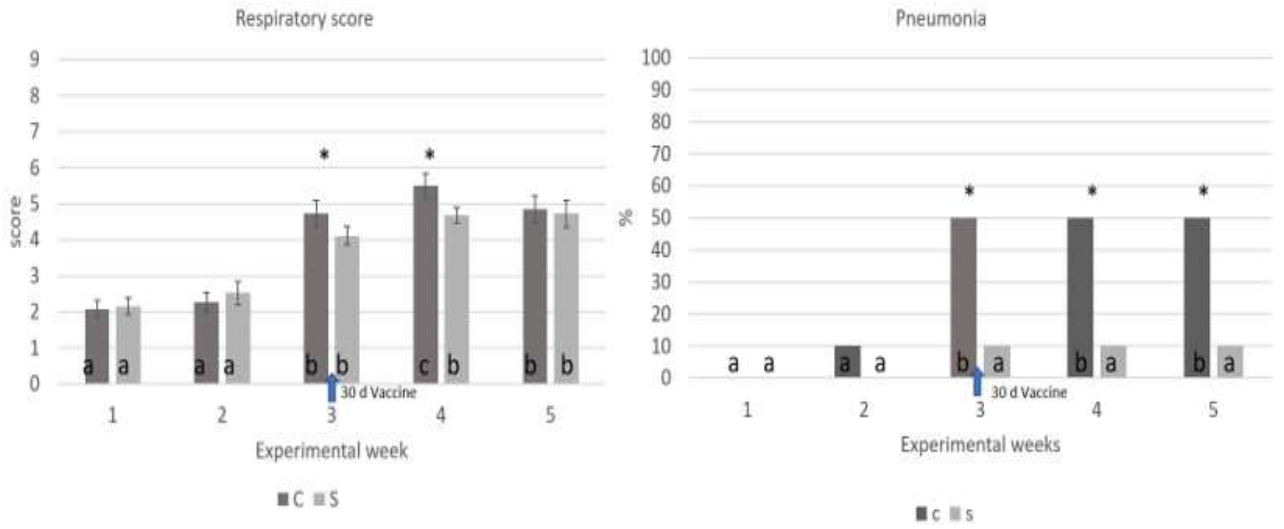

Figure 2. Sum of the respiratory score (median and 95% confidence interval) and pneumonia frequency of calves supplemented (S) or not (C) with Yeast.
* Indicates difference between treatments, a,b: different letters in the same treatment group indicate a significant difference between time, chi-squared test P ≤0.05.

Of the blood leukocyte measures, a reduction in neutrophilic oxidative metabolism was observed for group C when the calves were 33 days old compared to the other time points and in relation to group S (P=0.007 and P=0.001, respectively, figure 3). In the same period, there was an increase in the neutrophil:lymphocyte ratio 3 days after vaccination in both groups (C: P=0.05; S: P=0.09). From this period on, there was a high dispersion of data in the groups, as seven animals from group C and three from group S had a neutrophil:lymphocyte ratio >30%, with no significant difference between groups or between times (Figure 3).

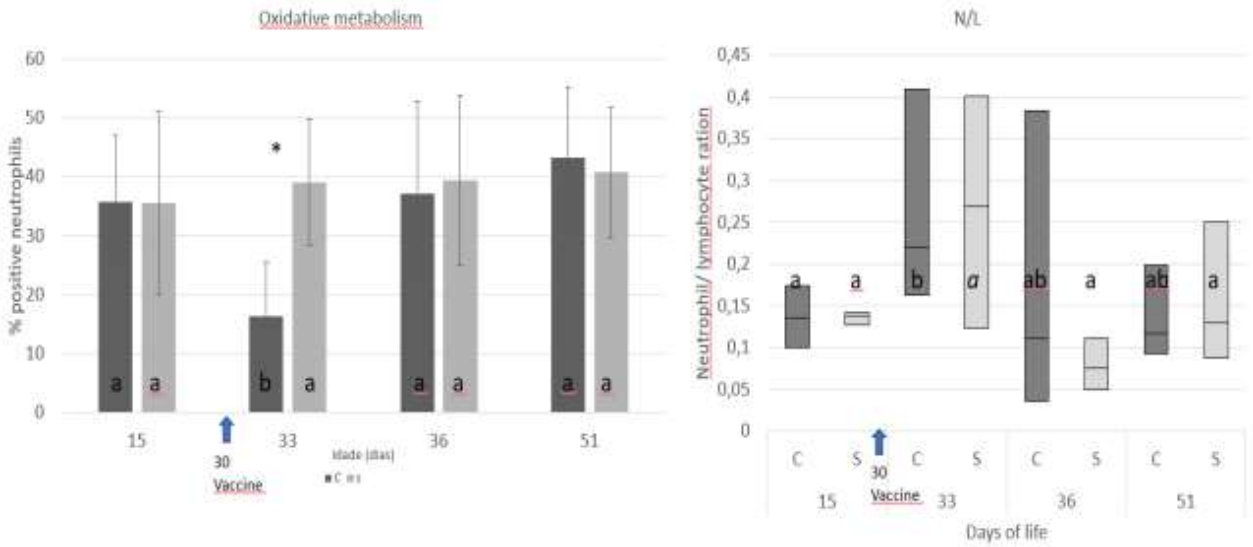

Figure 3. Blood neutrophils from calves supplemented (S) or (C) without Yeast: A. Neutrophilic oxidative metabolism (mean and standard deviation). B- neutrophil:lymphocyte ratio (median, 1st and 3rd quartiles).
* Indicates difference between treatments, P ≤0.05; Tukey's test for oxidative metabolism and Dunn's test for N/L ratio. *a,b* Different letters in the same treatment group indicate a significant difference between times, P≤0.05, parametric t test for oxidative metabolism and nonparametric t test for N/L ratio.a,b indicate a trend toward significant difference, p<0.10, t test between treatments.

In the respiratory tract, we observed that the MLV vaccine was a challenge to calves because it elicited respiratory signs in almost all calves, as reported by Grey et al. (2019) and Rossi et al. (2021). The vaccine mimics a mild viral infection that can promote increased local irritation and reduced phagocyte function within 3 and 7 days after vaccination, the period before antibody production, which makes the lung more susceptible to bacterial infections, especially in the first 3 days after vaccination (Rossi

et al., 2021), which is consistent with the findings of this study, in which inflammation after vaccination was also higher in group C, 3 days after the vaccination, when the calves were 33 days of life.

Oxidative metabolism is the production of intracellular reactive oxygen species by phagocytes, whose main function is eliminating engulfed pathogens (McGill & Sacco, 2020). Thus, the reduction of this function in group C, 3 days after vaccination, indicated a lower efficiency of the calves' phagocytes, which increased their susceptibility to bacterial diseases, especially under the highly challenging conditions of this experiment, in which the animals had immaturity immune and diarrhea. Due to these considerations, Chamorro and Palomares (2020) do not advocate for the administration of this vaccine in debilitated or stressed animals. Similarly, Martínez et al. (2023) propose intranasal BRSV vaccination in calves after one month of age to mitigate interference from maternal immunity and to elicit durable local and systemic immune responses. This recommendation contrasts with the findings of Woolums et al. (2004), who previously demonstrated the efficacy of intranasal BRSV vaccination in calves, even in those with maternal antibodies. Immunohistochemical analysis conducted by Martínez et al. (2023) revealed alterations in T and B cell distribution, with a notable emphasis on lung-associated lymphoid tissues and tonsils, underscoring the importance of local immune responses in their study.

Figure 4 shows the serum haptoglobin data. After vaccination, at 33 days of age, the median serum haptoglobin level was lower in group S than in group C (P=0.05), with no interaction between time.

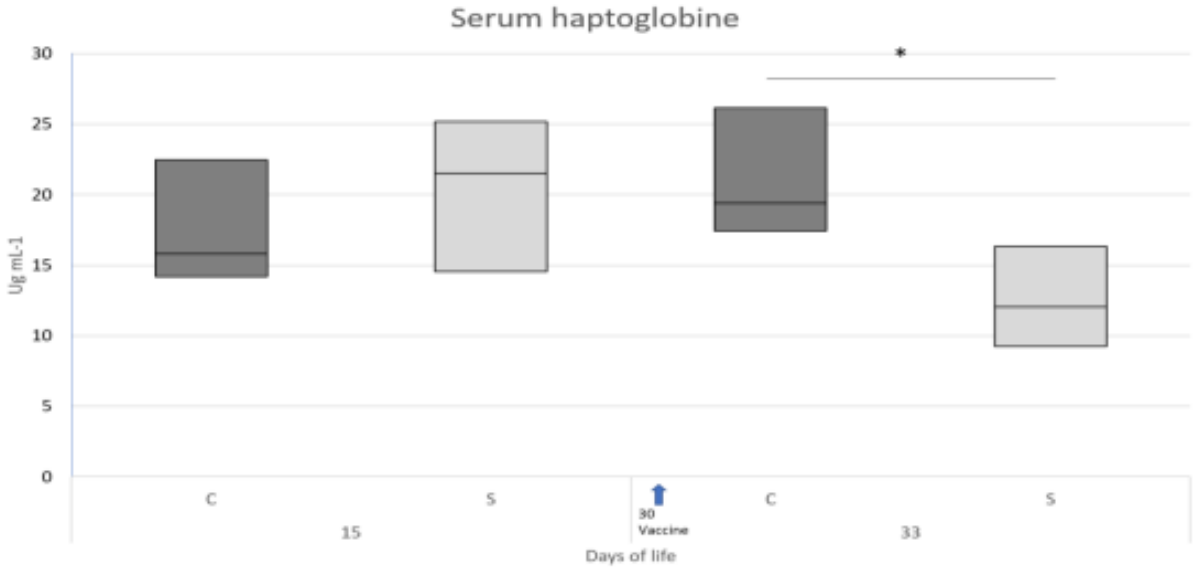

Figure 4. Serum haptoglobin (median, 1st and 3rd quartiles) of calves supplemented (S) or (C) without Yeast
* Indicates difference between treatments, P≤0.05, nonparametric t test.

Taube et al. (2024) observed that administering the same vaccine in a single dose to beef cattle upon entry to the feedlot resulted in heightened indicators of respiratory disease, including increased purulent nasal secretions, elevated orbital temperature, and heightened serum haptoglobin levels compared to unvaccinated animals. It is crucial to acknowledge that the immune response operates within a complex network of elements. Our study focused solely on two specific components: the oxidative metabolite of neutrophils and serum immunoglobulin production. It is plausible that a decline in one aspect of innate immunity may be offset by an increase in another immune response not examined in this study. Despite this limitation, more calves in group C developed pneumonia, a factor supported by changes in pulmonary auscultation, fever, increase of neutrophil:lymphocyte ratio, and increase of serum haptoglobin 3 days after vaccination, indicating that the animals presented an infectious condition, whether related to diarrhea, pneumonia, or both. Although subclinical pneumonia may not be diagnosed by white blood cell counts or pulmonary auscultation, serum haptoglobin is an early indicator of inflammation and is proportional to the extent of tissue injury (Decaris et al., 2022); thus, its reduction in group S indicated a less inflammatory status than in group C.

Inflammation was attenuated in the group S because β-glucans and mannan oligosaccharides increased the phagocytic response, which promoted an earlier elimination of pathogens and did not perpetuate the inflammation. The same was observed in the study by Virmond et al. (2020), in which the oxidative metabolism of blood phagocytes increased and serum haptoglobin decreased in feedlot-finished heifers supplemented with *Yeast* in fermentation culture medium, which reduced the occurrence of BRD. Also, Mahmoud et al. (2020) and McDonald et al. (2021) observed Yeast in fermentation culture medium attenuated the pneumonia in neonatal calves challenged with BRSV, but it did not influence the oxidative metabolism of blood neutrophils of this calves.

Although all the aforementioned studies found that *S cerivisiae* decrease the occurrence of BRD, there was a difference in the neutrophilic response capacity, possibly because the yeast presentation or dosage used, as well as because the magnitude of the challenge faced by the animals. These findings emphasize the importance of the quality of the different commercial products based on *S cerivisiae,* which produce a greater or a lesser effect than others, according to the quality and quantity of their constituents (Elghandour et al., 2022).

Figure 5 shows the data of serum immunoglobulins. Serum IgG at 51 days old tended to be greater in group S than group C (P=0.08), with no interaction between time. Serum IgA increased in group S at 51 days over group C and over the initial value (P=0.03 and 0.006, respectively).

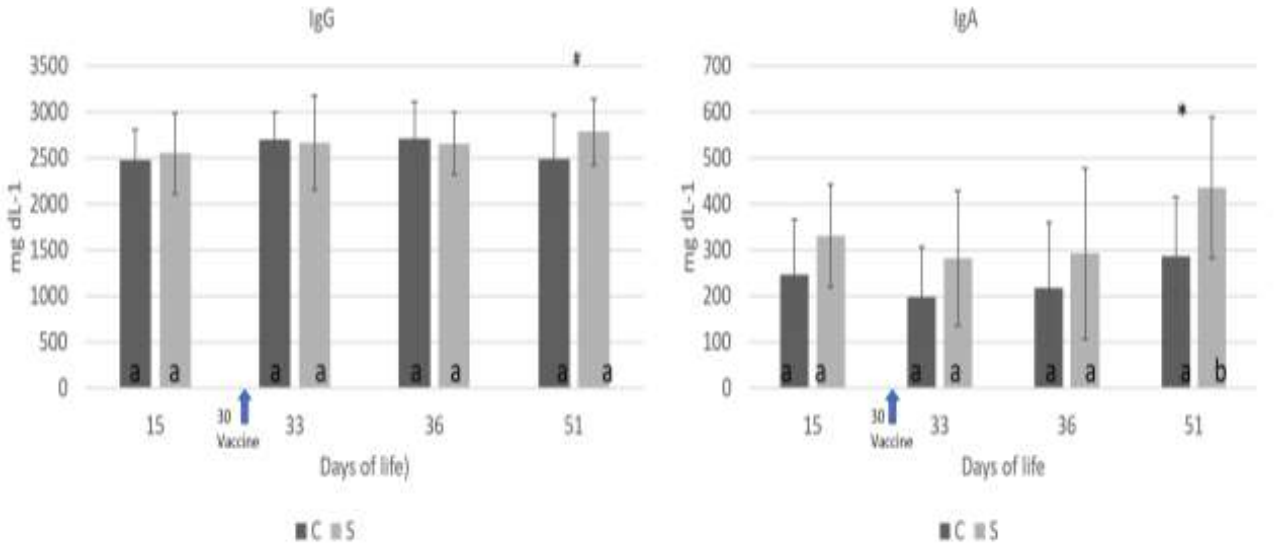

Figure 5. Serum immunoglobulins (means and standard deviation) in calves supplemented (S) or not (C) with the Yeast.
* Indicates difference between treatments, P ≤0.05; # trend toward significant difference between treatments, P≤0.10 Tukey's test,; a,b: different letters in the same treatment group indicate significant difference between times, P≤0.05, t test.

Regarding humoral immunity, the vaccination did not promote a significant increase in serum IgG in either group or in IgA serum in group C 21 days after vaccination, contrary to the findings of Ollivett et al. (2018) in neonatal calves, in which both serum immunoglobulins increased after vaccination. Importantly, we and Ollivett et al. (2018) did not measure the specific production of either immunoglobulin for each vaccine virus, but the increase of this specific titters could increase the total class of immunoglobulins. Rossi et al. (2021) also did not find an increase in bronchoalveolar IgG production of calves vaccinated against BRD, since this route of vaccination mainly stimulates the production of IgA in the mucosal sites and not necessarily in the blood, which could explain its stability in the immunoglobulin's serum of group C.

As the serum IgA production only increased when the calves were 51 days old and the supplemented calves started improving the diarrhea in the second week of the experiment, the question remains whether the greater intestinal and respiratory tract health was attributed only to the increase of the cellular immunity, or if there was also improvement in humoral immunity early only in the mucosal sites.

Group S showed greater weekly weight gain than group C at weeks 3 and 5 (P=0.05 and P=0.03, respectively) and greater live weight from the 3rd week of life on, which corresponded to the 28th day of the calves' old (P<0.05) (Figure 6). According to Thaler Neto et al. (2014), the greater intestinal immunity resulted in attenuation of the diarrhea, which interacted with the weight gain of the calves. In our study, the

lower intestinal and pulmonary inflammation of the animals resulted in a better intestinal absorptive capacity and a lower energy expenditure for immune response, which contributed to higher weight gains.

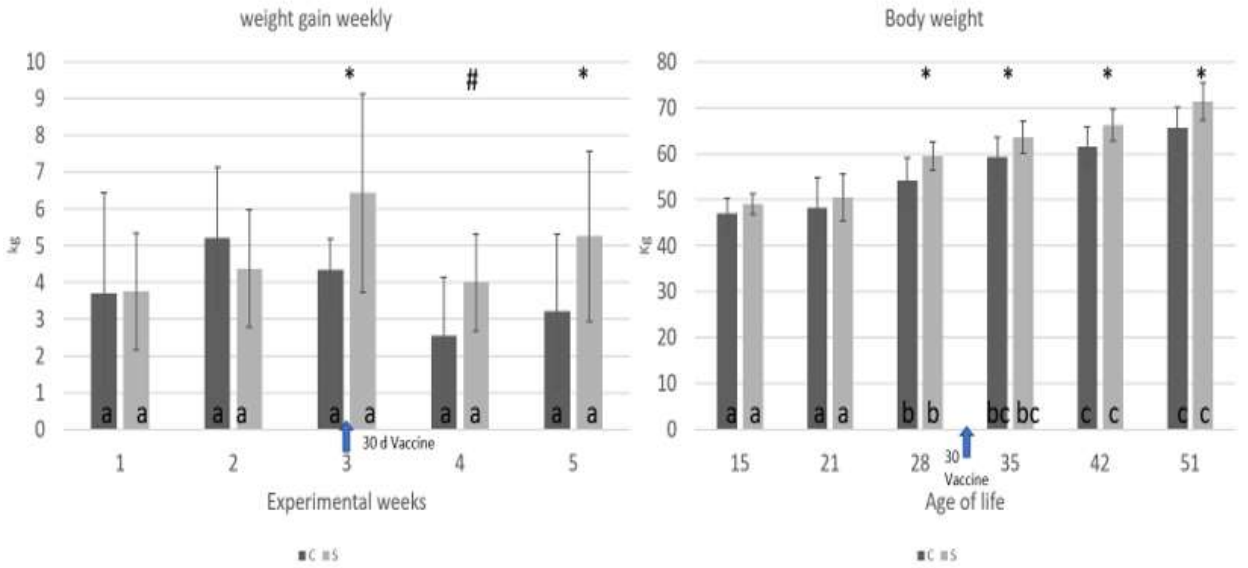

Figure 6. Weekly weight gain and live body weight (mean and standard deviation) of calves supplemented (S) or not (C) with Yeast.
* Indicates difference between treatments, P ≤0.05; # trend toward a significant difference between treatments, P≤0.10, Student's t test.
a,b Different letters in the same treatment indicate significant differences between time, Dunn's test P ≤0.05

Regarding diseases, there was no interference with the protocol used by the producer on the farm, who always applied oral fluid when the animal showed apathy and dehydration >5%. This protocol was applied to 8 animals in group C and 3 animals in group S. When the animals presented fever and respiratory signs, enrofloxacin was administered. This protocol was applied to 7 animals from group C and 4 animals from group S. These treatments generated an expenditure 68% higher for group C than for group S (Figure 7).

Regarding treatment costs, the choice of protocols was made by the farmer choice, which may vary between different husbandry systems. Even so, group C had more sick animals than group S, generating savings under any criteria for the use and choice of drugs.

Although our findings show that supplementation with autolyzed yeast increases the immunity of immunologically immature calves with comorbidities and that it attenuates the side effects of the intranasal vaccine containing attenuated BRD virus, it remains unknown whether this same supplementation will do the same effects in healthy calves.

Electrolyte and energy replenisher (diarrhea control)		
Protocol with 2L / 1 sachet	Control group	RumenYeast®
US$/protocol (US$/sachet)	3,23	3,23
Number of protocols used	51	9
Number of treated animals	8	3
Total solution cost, US$	164,73	29,07
Number of animals in the group	10	10
Prorated cost by group, US$/animal	16,47	2,91

Labor (respiratory conditions)		
	Control group	RumenYeast®
US$/treated animal	13,00	13,00
Number of treated animal	20	8
Total costs with labor US$	260,00	104,00
Number of animals in the group	10	10
Prorated cost by group, US$/animal	26,00	10,40

Eimeriosis		
All treated animals, therefore, no difference in cost between groups	Control group	RumenYeast®
Protocol with Toltrazuril (Bycox) 1 dose (D0) e Sulfadoxine (Borgal) 2 doses, (D0 e D15)		

Total costs (respiratory, hydration and labor)		
	Control group	RumenYeast®
US$/total	451,60	143,80
Number of animals in the group	10	10
Prorated cost by group, US$/animal	45,16	14,38

RumenYeast= Autolized *S cerivisiae** The value of the dollar on the quotation day was 1 real = US$5.23 - Banco do Brasil, 5th august, 2023.

Figure 7. Cost of health care for calves naturally infected with Eimeria sp. and vaccinated against BRD.

Conclusion

Autolyzed yeast demonstrated a capacity to mitigate the inflammatory response associated with Eimeriosis and vaccine reaction (BRD), consequently enhancing weight gain in immunologically immature calves. These findings provide evidence suggesting that autolyzed yeast supplementation may contribute to an overall improvement in the health status of calves.

Acknowledgments

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

References

Atkinson, D. J., Keyserlingk, M. A. G., & Weary, D. M. (2017). Benchmarking passive transfer of immunity and growth in dairy calves. *Journal of Dairy Science, 100*(5), 3773-3782. doi: 10.3168/jds.2006-11800

Bertagnon, H. B., Depaoli, C. R., Oliveira, S. N., Milla, B., Zdepski, B. F., & Garbossa, G. (2024). Immunostimulation of bronchoalveolar response in calves vaccinated against bovine respiratory disease. *Pesquisa Veterinária Brasileira, 44*(1), 1-8 e07374. doi: 10.1590/1678-5150-PVB-7374

Chamorro, M. F., & Palomares, R. A. (2020). Bovine respiratory disease vaccination against viral pathogens modified-live versus inactivated antigen vaccines, intranasal versus parenteral, what is the evidence? *Veterinary Clinical Food Animal, 36*(2), 461-472. doi: 10.1016/j.cvfa.2020.03.006

Ciarlini, P. C., Ciarlini, L. D. R. P., Alencar, N. X., Hohayagawa, A., & Rodrigues, C. F. C. (2002). Metabolismo oxidativo de neutrófilos em ovelhas naturalmente infectadas por nematódeos gastrintestinais e correlação entre nível sérico de cortisol e carga parasitária. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia, 54*(2002), 242-247. doi: 10.1590/S0102-09352002000300004

Decaris, N., Buczinski, S., Tárdon, D. I. C., Camargo, L., Schllemer, N. R., Hagen, S. C. F., Woolums, A., & Gomes, V. (2022). Diagnostic accuracy of Wisconsin and California scoring systems to detect bovine respiratory disease in preweaning dairy calves under subtropical environmental conditions, *Journal of Dairy Science, 105*(9), 7750-7763. doi: 10.3168/jds.2021-21491

Elghandour, M. M. M., Abu Hafsa, S. H., Cone, J. W., Salem, A. Z. M. S., Anele, U. Y., & Alcala-Canto, Y. (2022). Prospect of yeast probiotic inclusion enhances livestock feeds utilization and performance: an overview. *Biomass Conversion and Biorefinery, 14*(1),2923-2935. doi: 10.1007/s13399-022-02562-6

Fomenky, B. E., Do, D. N., Talbot, G., Chiquette, J., Bissonnette, N., Chouinard, Y. P., Lessard, M., & Ibeagha-Awemu, E. M. (2018). Direct-fed microbial supplementation influences the bacteria community composition of the gastrointestinal tract of pre-and post-weaned calves. *Scientific Reports, 8*(1), 1-21. doi: 10.1371/journal.pone.0061217

Flores, G. V. B., Thomaz, G. R., Willi, H., Neto, Rossi, P. S., Strickler, F., & Bertagnon, H. G. (2019). Effect of Enterococcus faecium and Saccharomyces cerevisiae on immune response, hematological parameters and weight gain of calves fed corn silage. *Veterinary and Animal Science, 26*(1), 1-11. doi: 10.35172/rvz.2019.v26.353

Grey, D., Ellis, J. A., Gow, S. P., Lacoste, S. R., Allan, G. M., & Mooney, M. H. (2019). Profiling of local disease-sparing responses to bovine respiratory syncytial virus in intranasally vaccinated and challenged calves. *Journal of Proteomics, 204*(1), 1-12. doi: 10.1016/j.jprot.2019.103397

Lopez-Osorio, S., Villar, D., Failing, K., Taubert, A., Hermosilla, C., & Chaparro-Gutierrez, J. J. (2020). Epidemiological survey and risk factor analysis on Eimeria infections in calves and young cattle up to 1 year old in Colombia. *Parasitology Research, 119*(1), 255-266. doi: 10.1007/s00436-019-06481-

Mahmoud A. H. A., Slate, J. R., Hong, S., Yoon, I., & McGill, J. L. (2020). Supplementing a Saccharomyces cerevisiae fermentation product modulates innate immune function and ameliorates bovine respiratory syncytial virus infection in neonatal calves. *Journal of Animal Science, 98*(8), skaa252. doi: 10.1093/jas/skaa252

Martínez, D. A., Chamorro, M. P., Passler, T., Huber, L., Walz, P. H., Thoresen, M., Raithel, G., Silvis, S., Stockler, R., & Woolums, A. R. (2023). Local and systemic antibody responses in beef calves vaccinated with a modified-live virus bovine respiratory syncytial virus (BRSV) vaccine at birth following BRSV infection. *Veterinary Sciences, 10*(1), 20-33. doi: 10.3390/vetsci10010020

McDonald, P. O., Schill, C., Maina, T. W., Samuel, B., Porter, M., Yoon, I., & McGill, J. L. (2021). Feeding Saccharomyces cerevisiae fermentation products lessens the severity of a viral-bacterial coinfection in preweaned calves. *Journal Animal Science, 99*(11), skab300. doi: 10.1093/jas/skab300

McGill, J. L., & Sacco, R. E. (2020). The immunology of bovine respiratory disease: recent advancements. *Veterinary Clinics of North America: Food Animal Practice, 36*(2), 333-348. doi: 1016/j.cvfa.2020.03.002

Oelschlaeger, T. A. (2010). Mechanisms of probiotic actions - a review. *Internal Journal Medical Microbiology, 300*(1), 57-62. doi: 10.1016/j.ijmm.2009.08.005

Ollivett, T. L., Leslie, K. E., Duffield, T. F., Nydam, D. V., Hewson, J., Caswell, J., & Kelton, D. F. (2018). Field trial to evaluate the effect of an intranasal respiratory vaccine protocol on calf health, ultrasonographic lung consolidation, and growth in Holstein dairy calves. *Journal of Dairy Science, 101*(9), 8159-8168. doi: 10.3168/jds.2017-14271

Ollivett, T. L., Nydam, D. V., Bowman, D. D., Zambriski, J. A., Bellosa, M. L., Linden, T. C., & Divers, T. J. (2009). Effect of nitazoxanide on cryptosporidiosis in experimentally infected neonatal dairy calves. *Journal of Dairy Science, 92*(4), 1643-1648. doi: 10.3168/jds.2008-1474

Rossi, P. S., Mattei, R. I., Schllemer, N. R., Thomaz, G. R., Antunes, A. V., Virmond, M. P., Taube, M. J., & Bertagnon, H. G.

(2021). The effect of bovine vaccines against respiratory viruses administered either intranasal or intramuscular on broncho-alveolar fluid cells of heifers. *Veterinary Quarterly, 41*(1), 97-106. doi: 10.1080/01652176.2020.1870019

Thaler, A., Neto, Gomes, I. P. O., Dias, A. L. G., Córdova, H. A., & Dal Pizzol, J. G. (2014). Desempenho de bezerros da raça holandesa suplementados com probiótico a base de Saccharomyces cerevisae, cepa Ka500 e Pediococcus Acidilactici. *Archives of Veterinary Science, 19*(4), 10-16. doi: 10.5380/avs.vl9i4.34836

Taube, M. J., Rossi, P. S., Sague, A. H., Flores, G. V. B., Peres, J. A., Garbossa, G., & Bertagnon, H. G. (2024). Intranasal and intramuscular commercial vaccines against respiratory viruses in steers finished in feedlot. *Acta Veterinaria Brasílica,* (In press).

Uyeno, Y., Shigemori, S., & Shimosato, T. (2015). Effect of probiotics/prebiotics on cattle health and productivity. *Microbes and Environment, 30*(2), 126-132. doi: 10.1264/jsme2.ME14176

Villot, C., Chen, Y., Pedgerachny, K., Chaucheyras-Durand, F., Chevaux, E., Skidmore, A., Guan, L., & Steele, M. A. (2020). Early supplementation of Saccharomyces cerevisiae boulardii CNCM I-1079 in newborn dairy calves increases IgA production in the intestine at 1 week of age. *Journal of Dairy Science, 103*(9), 8615-8628. doi: 10.3168/jds.2020-18274

Virmond, M. P., Rossi, P. S., Antunes, A. V., Mattei, R. I., Schllemer, N. R., Thomaz, G. R., & Bertagnon, H. G. (2020). Fresh yeast additives improve immune parameters and reduce respiratory disease in heifers finished in feedlots. *Semina: Ciências Agrárias, 41*(6), 3177-3188. doi: 10.5433/1679-0359.2020v41n6Supl2p3177

Woolums, A. R., Brown, C. C., & Brown, J. C., Jr. (2004). Effects of a single intranasal dose of modified-live bovine respiratory syncytial virus vaccine on resistance to subsequent viral challenge in calves. *American Journal of Veterinary Research, 65*(3), 363-372. doi: 10.2460/ajvr.2004.65.363