PESQUISA VETERINÁRIA BRASILEIRA
Brazilian Journal of Veterinary Research
ISSN 0100-736X (Print)
ISSN 1678-5150 (Online)

Pesq. Vet. Bras. 44:e07374, 2024
DOI: 10.1590/1678-5150-PVB-7374

Original Article
Livestock Diseases

Immunostimulation of bronchoalveolar response in calves vaccinated against bovine respiratory disease[1]

Heloisa G. Bertagnon[2]*, Caroline R. Depaoli[2], Sarah N. Oliveira[2], Bianca Milla[2], Bruna F. Zdepski[2] and Gabriela Garbossa[2]

ABSTRACT.- Bertagnon H.G., 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:e07374, 2024*. Departamento de Medicina Veterinária, Universidade Estadual do Centro-Oeste, Rua Simeão Varela de Sá 3, Vila Carli, Guarapuava, PR 85040-080, Brazil. E-mail: hbertagnon@unicentro.br

Although intranasal bovine respiratory disease (BRD) vaccines containing live attenuated virus elicit greater stimulation of local humoral immunity response, they can mimic a viral infection, responsible for reducing innate defense during the establishment of vaccine-induced immunity. Probiotics containing *Saccharomyces cerevisiae* or *Enterococcus faecium* reduced the occurrence of BRD in neonatal calves challenged with the bovine respiratory syncytial virus (BRSV). Furthermore, the probiotics potentiated the humoral immune response after vaccination in murine models, raising the question of whether they could have the same effect in calves. This study aimed to verify if the probiotic containing *E. faecium* and *S. cerevisiae* attenuates the inflammation caused by the vaccine against BRD in the respiratory tract region in calves. Twenty-four healthy Jersey calves, aged 6 to 7 months old, were divided into the groups: control (C), supplemented (S), vaccinated (V), and supplemented and vaccinated (SV), with six animals in each of them. Supplemented groups (S and SV) received *S. cerevisiae* and *E. faecium* once a day on D-15 for 51 days (Probios precise®, Ouro Fino®, 2g/day/animal). Vaccinated groups (V and SV) received a single dose of an intranasal BRD vaccine on day 0 (Inforce®, Zoetis®, 1ml/ nostril). The control group was not supplemented or vaccined. Irritation of the respiratory tract and bronchoalveolar (BA) evaluations: cytology, phagocyte function, and IgA were measured on D-15, D3, D7, and D21. The vaccinated groups showed greater irritation of the nasopharynx and trachea. However, only Group V showed a reduction in BA phagocyte function and an increase in cellularity by a neutrophil influx in the BA region. Regarding IgA BA, SV showed the greatest increase, followed by S and V, concerning C. We conclude that isolated supplementation with *E. faecium* and *S. cerevisiae* promoted increased production of BA IgA. In association with the vaccine, the supplementation attenuated the inflammation of the respiratory tract produced by the vaccine itself, avoiding the reduction of phagocyte function BA, besides potentiating the humoral immune response of the vaccine containing live attenuated virus against BRD.

INDEX TERMS: Alveolar macrophage, reactive oxygen species, IgA, bronchoscopy, pneumonia, calf.

RESUMO.- [Imunoestimulação da resposta broncoalveolar de bezerros vacinados contra complexo respiratório bovino.] Embora vacinas intranasais contra complexo respiratório bovino (CRB) contendo vírus vivo atenuado provoquem maior estimulação de reposta humoral local, elas podem mimetizar uma infecção viral responsável por reduzir a defesa imune inata durante o estabelecimento da imunidade vacinal. Probióticos contendo *Saccharomyces cerevisiae* e *Enterococcus faecium* reduziram a ocorrência de CRB em bezerras neonatas desafiados com vírus respiratório sincicial bovino e potencializaram a resposta humoral após a vacinação em modelos murino, gerando-se a dúvida se poderiam ter o mesmo efeito em bezerros. Este trabalho teve o objetivo de verificar se o probiótico contendo *E. faecium* e *S. cerevisiae* atenua a inflamação causada pela vacina na região do trato respiratório de bezerras. Vinte e quatro bezerras da raça Jersey,

[1] Received on August 17, 2023.
Accepted for publication on October 22, 2023.
[2] Departamento de Medicina Veterinária, Universidade Estadual do Centro-Oeste (UNICENTRO), Rua Simeão Varela de Sá 3, Vila Carli, Guarapuava, PR 85040-080, Brazil. *Corresponding author: hbertagnon@unicentro.br

sadias com idade entre 6 a 7 meses de idade foram divididas nos grupos: controle (C), suplementado (S), vacinado (V) e suplementado e vacinado (SV), com seis animais em cada. Os grupos suplementados (S e SV) receberam *S. cerevisiae* e *E. faecium* uma vez ao dia, no dia D-15, durante 51 dias (Probios precise®, Ouro Fino®, 2g/dia/animal). Os grupos vacinados (V e SV) receberam a vacina intranasal contra CRB em dose única no dia 0 (Inforce®, Zoetis®, 1ml/ narina). Mensurou-se a irritação do trato respiratório e os parâmetros broncoalveolares (BA): citologia, função de fagócitos e IgA, nos dias D-15, D3, D7 e D21. Os grupos vacinados apresentaram maior irritação da nasofaringe e traqueia, porém apenas o V apresentou redução da função dos fagócitos BA e aumento da celularidade por influxo neutrofílico. Em relação a IgA BA, o SV apresentou o maior aumento, seguido do S e do V, em relação ao C. Conclui-se que a suplementação com *E. faecium* e *S. cerevisiae* isolada promoveu aumento de produção de IgA BA. Em associação com a vacina, atenuou a inflamação do trato respiratório produzida pela mesma, evitando a redução da função de fagócitos BA, além de potencializar a resposta humoral da vacina contendo vírus vivo atenuado contra o CRB.

TERMOS DE INDEXAÇÃO: Macrófago alveolar, espécies reativas de oxigênio, IgA, broncoscopia, pneumonia, bezerros.

INTRODUCTION

Bovine respiratory disease (BRD) is a condition of multifactorial origin related to a complex interaction between environmental stressors, immune susceptibility of the animals, and viral respiratory pathogens such as bovine herpes virus-1 (BHV-1), bovine parainfluenza virus-3 (BPIV-3), bovine respiratory syncytial virus (BRSV), and bovine viral diarrhea virus (BVDV) associated or not with bacteria *Mannheimia haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus.* Its high incidence worldwide has motivated the development of numerous preventive protocols, highlighting vaccines as one of the most assertive measures for controlling BRD due to their economic advantages and because they are free of bacterial resistance compared to prophylactic antibiotics (McGill & Sacco 2020).

Most commercial vaccines are targeted against the viral agents BHV-1, BPIV-3, BRSV, and BVDV, the first three in the attenuated form and the last one inactivated. These vaccines are intended for administration via injection, with a maximum level of immune protection between 35 and 40 days after the first vaccination (Fulton 2015, Baccili et al. 2019). Meanwhile, the Brazilian commercially available intranasal vaccines contain live attenuated viruses such as BHV-1, BPIV-3, and BRSV or only the BRSV. This alternative is the best for dairy calves, including immunologically immature ones, since it promotes a superior local immune response earlier than systemic vaccination (Chamorro & Palomares 2020, Rossi et al. 2021).

However, Dou et al. (2015) and Rossi et al. (2021) suggested that this vaccine mimics a tenuous viral infection by inoculating live attenuated viruses directly into the respiratory tract. According to the authors, this promotes both viral eliminations by the vaccinated animals and reduced oxidative metabolism of alveolar macrophages until seven days after the vaccination. Thus, the question arises whether this protocol may increase the susceptibility of the respiratory tract to secondary bacterial infections during the establishment of vaccine immunity, which occurs between 7 and 21 days.

Possibly, this is the reason why Fulton (2009) reported that the vaccine prevented BRD in calves only partially when there was bacterial involvement, which motivates the study of strategies to mitigate the side effects of the vaccine as well as potentiate the vaccine immune response in animals. In this regard, probiotic supplementation may function as an immune-stimulatory, given that both *Enterococcus faecium* and *Saccharomyces cerevisiae* stimulate the cellular and humoral immune response of cattle 15 days after the supplementation (Qadis et al. 2014, Flores et al. 2019, Mahmound et al. 2020).

Thus, we questioned whether supplementation with *E. faecium* and *S. cerevisiae* could provide any benefit on cell function and IgA production in the respiratory tract of young, healthy, pasture-raised calves vaccinated against BRD by intranasal vaccine containing a live attenuated virus.

MATERIALS AND METHODS

Animal Ethics. This research was approved by the local Animal Ethics Committee/UNICENTRO (038/2020).

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

The farm had 30 calves aged 8±2 months and weighing 150±20kg, without previous vaccination and with negative results for BVDV (ELISA test BVDV antigen®, Idexx, São Paulo, Brazil). Twenty-four calves were selected by the inclusion criteria: healthy animals based on hemogram, physical examination, and bronchoscopic evaluation. The animals were dewormed 30 days before the experiment. They remained in the same oat grass (*Avena sativa*) pasture and ryegrass (*Lolium multiflorum*) without cover and with water supply *ad libitum*. Twice a day, they were individually fed in tie-stalls with corn silage *ad libitum*, with 5% of leftovers.

Experimental design. The experimental design was completely randomized, 2x2 factorial, blinded about treatment, containing four groups with six animals each, evaluated at four-time points, wherein each animal was an experimental unit. Treatments: V = vaccination with a 2ml dose of the commercial vaccine (Inforce 3®, Zoetis, Brazil) composed of infectious bovine rhinotracheitis (IBR) virus (strain RLB 106), PIV-3 (strain RLB 103, both with thermosensitive samples), and BRSV (strain 375, modified-live sample); S = animals received daily a 2g oral supplementation of probiotics containing *Enterococcus faecium* (Min. 2.5x109CFU/g) and *Saccharomyces cerevisiae* (Min. 1.0x109CFU/g) (Probios Precise®, Ouro Fino, São Paulo) for 51 days. The animals in the Control Group (C) did not receive any treatment, and the Group VS received both the vaccine and supplement.

On D-15 (before the vaccine administration), the supplementation with the probiotic started, administered once a day on top of 100g of corn silage in tie stalls, ensuring its consumption. The groups that were not supplemented received only 100g of silage. After

consumption, the remainder of the silage was provided. At D0, the animals were vaccinated according to their treatment distribution.

Samples collection and analyses. The physical examinations, blood collection, and bronchoalveolar lavage (BAL) were performed on days D-15, D3, D7, and D21 in relation to vaccination day.

In addition, 8ml of blood was collected from the external jugular vein to analyze the serum urea concentration in an enzymatic test with commercial kits (Urea UV Liquiform Vet®, Labtest®, Brazil). For the bronchoalveolar evaluations, the animals underwent both respiratory tract inspection and the collection of BA lavage. For this, the animals were sedated with xylazine (Anasedan®-cevaBRA, São Paulo, Brazil) at a dose of 0.02mg/kg intravenously. Moreover, the bronchoscopy was performed with a Fibro Gastroscope (XQ-20, olympus®, São Paulo, Brazil; dimensions: 1.0mm X 0.9mm) introduced through the nostrils until reaching the bronchial region, and there was no resistance to passage. In this approach, a score for nasopharyngeal and tracheal mucosa irritations was assigned: Score 1 = smooth, shiny, and normal colored mucosa; Score 2 = nasopharynx irritation (reddened mucous membranes, evidenced vessels, and irregularities); Score 3 = trachea irritation (reddened mucous membranes, evidenced vessels, and irregularities); and Score 4 = irritation both in nasopharynx and trachea.

After inspection of all regions, bronchoalveolar lavage was collected by instilling 30mL of sterile 0.9% saline solution at room temperature into each bronchus and then aspirated by 20mL syringe according to the technique described by Batista et al. (2012). The samples were then centrifuged at 1,000g for 15 minutes at 4°C, and the supernatant was separated from the cell pellet.

The urea concentration was measured in the supernatant using the enzymatic technique adapted for small concentrations using commercial kits (Urea UV Liquiform Vet®, Labtest®, Brazil). Moreover, IgA was measured by the ELISA technique (190516 IgA Cow ELISA Kit®, Abcam, Cambridge, United States) using a 1:4 dilution. Both analyses were performed in duplicate, with a coefficient of variation of less than 0.5%

In the cell pellet, BA cellularity was quantified in a Neubauer chamber. In addition, the cell profile was analyzed according to morphotintorial characteristics using light microscopy, and cellular oxidative metabolism was measured by the quantitative nitroblue tetrazolium test, as Rossi et al. (2021) described.

The values of immunoglobulins and cellularity obtained in the lavage were corrected according to blood urea using the formula used by Bertagnon et al. (2007).

VR = VE X (US/UL), with AV = actual value of immunoglobulins in lavage, RV = retrieved value for the immunoglobulins in lavage, US = urea concentration in blood serum, and UL = urea concentration in the lavage.

Statistical analysis. were conducted using the Statistical Analysis System (SAS) software version 9.3. The data were evaluated for normality (Kolmogorov-Smirnov test) and homogeneity (Barellett test). The BA data, like cytology, oxidative metabolism of bronchoalveolar, and IgA, were normal and homogenous and evaluated using parametric tests. The means or medians were compared. The irritation data were treated as non-parametric data and presented as medians and first and third quartiles. Each treatment was analyzed using the mixed model (PROC MIXED). The treatment was considered a fixed effect, and the time was considered an aleatory effect. If the variable showed statistical, Tukey or Dunn's test was performed to verify the period in which the treatment began to exert its effect. Statistical significance was set at $P<0.05$.

RESULTS

During the experiment, no animal showed abnormalities in the blood count, nor fever, apathy, or altered respiratory sounds. Retropharyngeal lymph node reactivity and mucopurulent secretion in the nostril and nasopharynx were observed from D3 onwards, especially in the animals in V and SV. While two animals from Groups C and S showed these changes on D3, four animals showed them on V and SV. On D7, only one animal from C and one from S had these manifestations, while three animals from V and SV had them. Furthermore, two animals from each group manifested these changes at D21.

These changes corresponded to the findings of nasopharyngeal and tracheal irritation observed by bronchoscopy, which had a statistical interaction of treatment but not of the time (Fig.1). At D3, vaccinated animals (V and SV) showed higher irritation scores than S ($P=0.05$), and at D7, these groups showed higher irritation than the unvaccinated ones ($P=0.05$).

Regarding the BA wash, there was much variation in its recovered volume, ranging from 5 to 25ml. Thus, the dilution of the samples was different in each collection; the absolute values of cellularity and IgA BA were calculated according to the concentration of blood urea and the BA wash.

The data concerning the oxidative metabolism of BA cells underwent interaction time and treatment ($P=0.001$) (Fig.2). Concerning treatment, the SV group showed higher oxidative metabolism of BA cells than the other groups (0,004). In the derivation of the data, the SV group showed higher oxidative metabolism of BA cells than the other groups on D3 ($P=0.03$) and higher than Group V on D7 ($P=0.05$). In the time interaction, this function remained stable in Group C, decreased in V on D3 and D7 ($P=0.04$), and increased in SV on D3 and D7 ($P=0.02$).

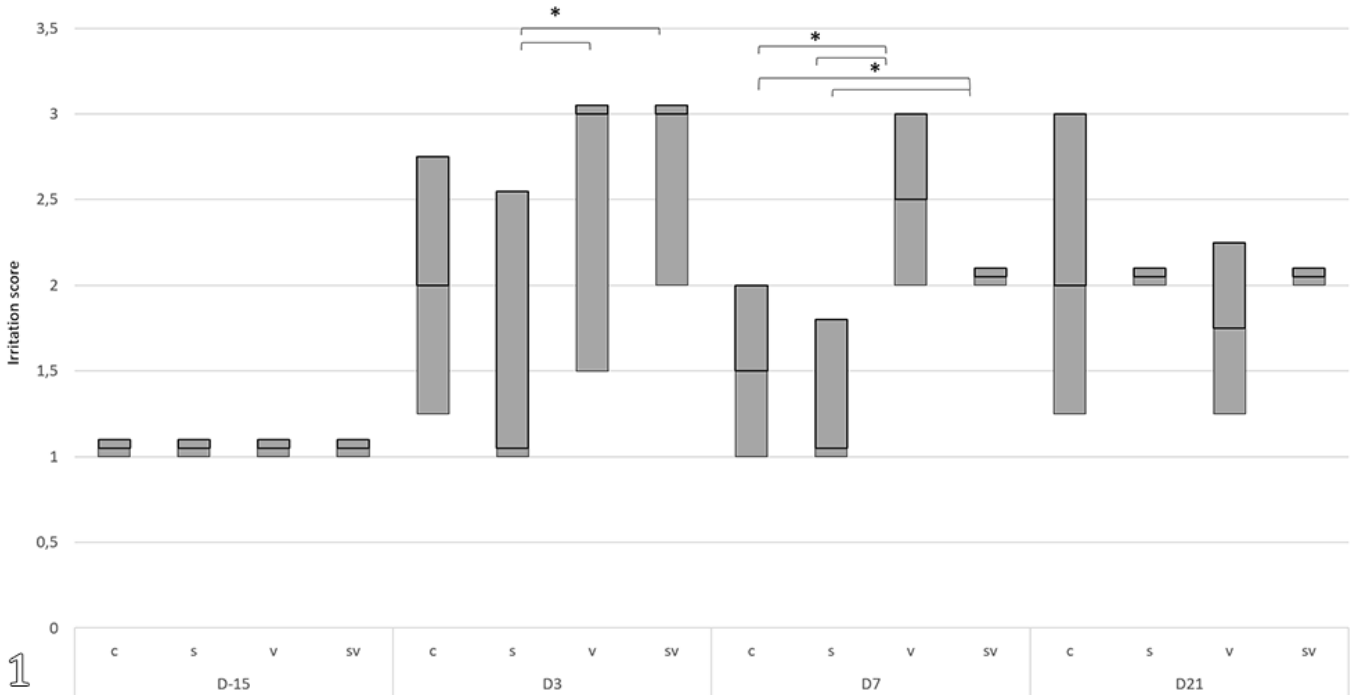
The data regarding the cellularity of the BA region are presented in Figure 3-6 and underwent time and treatment interaction ($P\leq0.05$). At the same time, Group V showed an increase in bronchoalveolar cells from D3 in the time interaction ($P<0.001$) and D3 and D21 in the treatment interaction in relation to C and S ($P\leq0.05$). SV only showed this increase on D21 in the time and treatment interaction ($P<0.001$) and the treatment interaction in relation to C and S ($P=0.05$). There was no statistical change for this variable in C and S.

Furthermore, Group V showed decreased alveolar macrophages and increased neutrophils at D3 compared to the other groups ($P\leq0.05$). At D21, Group V showed an increase in neutrophils relative to S and lymphocytes relative to C and S ($P\leq0.05$). In contrast, SV showed a reduction in alveolar macrophages relative to S ($P=0.04$) and an increase in neutrophils and lymphocytes relative to C and S ($P\leq0.05$).

IgA BA also had a time-treatment interaction ($P=0.006$). Regarding treatment, the SV group showed higher IgA BA than C ($P=0,005$). In the derivation of the data, IgA BA was higher in Group V compared to C and S and higher in SV compared to all groups at D21 ($P<0.05$). IgA BA increased in the S, V, and SV groups at D7 compared to the initial time points ($P<0.05$) and remained high at D21 for the V and SV groups ($P<0.05$) (Fig.7).

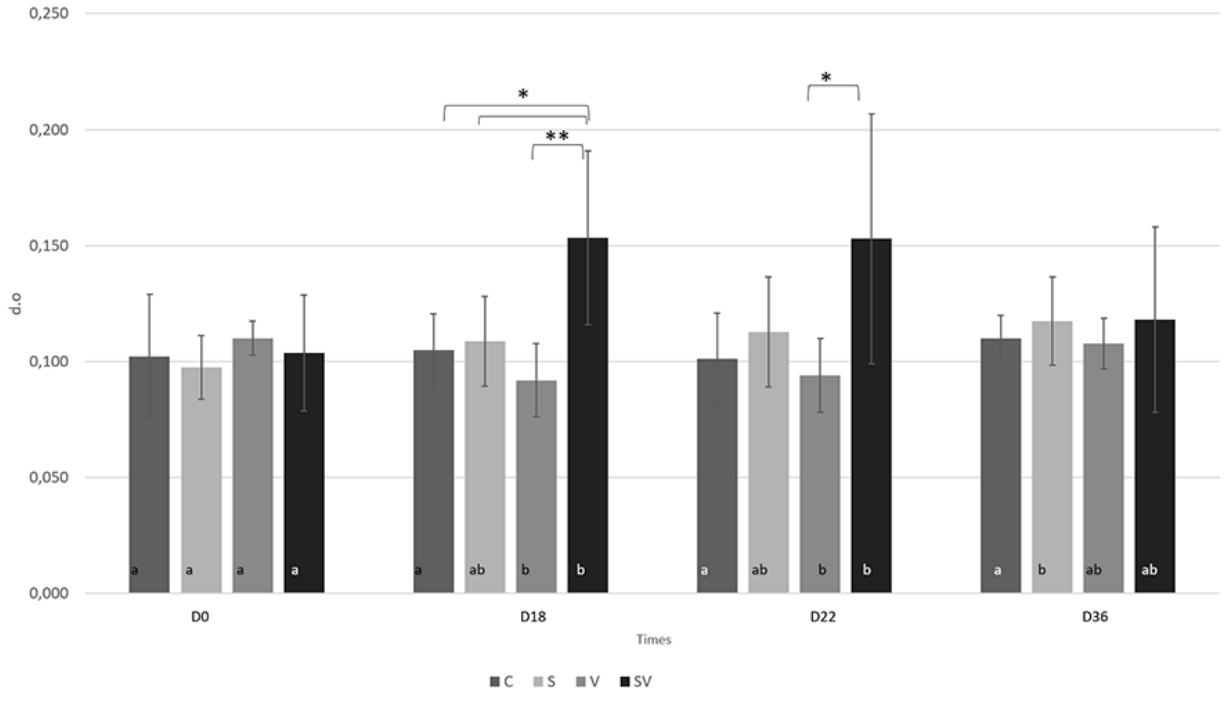
DISCUSSION

The intranasal vaccine containing live attenuated BRD virus promoted increased IgA production but reduced the phagocyte



1

Fig.1. Nasopharyngeal and tracheal irritation scores of healthy calves treated with the supplement, bovine respiratory disease (BRD) vaccine, or both. Control group (C), group supplemented with *Enterococcus faecium* and *Saccharomyces cerevisiae* (S), group with the intranasal vaccine (V), group vaccinated and supplemented (SV). Values expressed as median and quartiles 25 and 75%. Statistical difference $P \leq 0.05$, Dunn test (*). There was no time effect $P \leq 0.05$, Dunn test.



2

Fig.2. Oxidative metabolism of bronchoalveolar cells from healthy calves treated with the supplement, bovine respiratory disease (BRD) vaccine, or both. Control group (C), group supplemented with *Enterococcus faecium* and *Saccharomyces cerevisiae* (S), group with the intranasal vaccine (V), group vaccinated and supplemented (SV). Optical density (d.o.). Mixed model treatment $P=0.001$, statistical difference $P \leq 0.05$ (*), statistical difference $P \leq 0.01$ between treatments (**). Different lowercase letters on the bars indicate statistical differences for the same group at different times, $P \leq 0.05$, Tukey test.

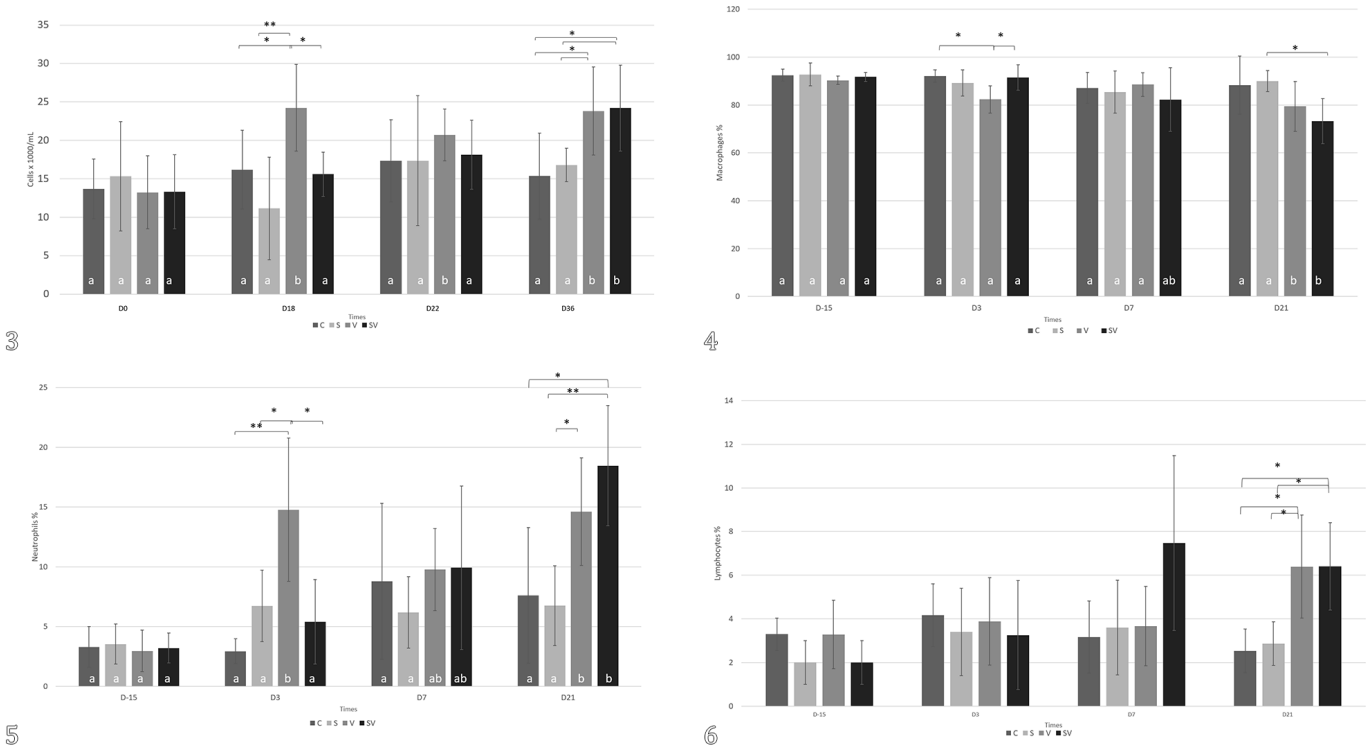


Fig.3-6. Total cells in the bronchoalveolar region of healthy calves treated with the supplement, bovine respiratory disease (BRD) vaccine, or both. (3) Total cellularity, (4) percentage of macrophages, (5) percentage of neutrophils, (6) percentage of lymphocytes. Control group (C), group supplemented with *Enterococcus faecium* and *Saccharomyces cerevisiae* (S), group with intranasal vaccine (V), group vaccinated and supplemented (SV). Mixed model treatment $P \leq 0.001$. Statistical difference $P \leq 0.05$ (*), statistical difference $P \leq 0.01$ between treatments (**). Different lowercase letters on the bars indicate statistical differences for the same group at different times, $P \leq 0.05$ Tukey test.

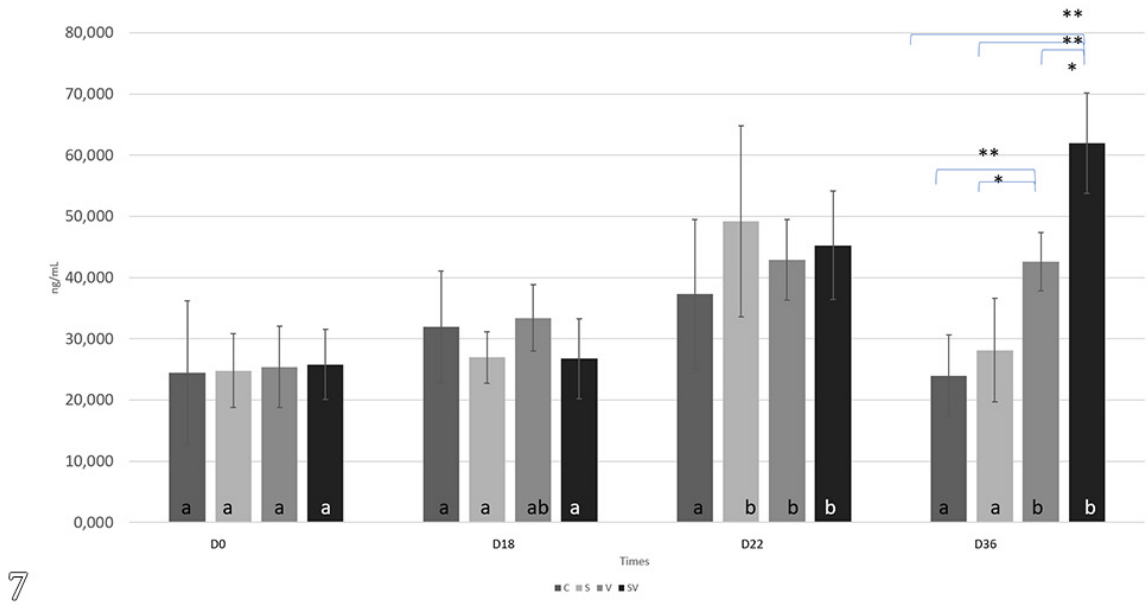


Fig.7. Bronchoalveolar IgA of healthy calves treated with the supplement, bovine respiratory disease (BRD) vaccine, or both. Control group (C), group supplemented with *Enterococcus faecium* and *Saccharomyces cerevisiae* (S), group with intranasal vaccine (V), group vaccinated and supplemented (SV). Mixed model treatment $P=0.006$. Statistical difference $P \leq 0.05$ (*), statistical difference $P \leq 0.01$ between treatments (**). Different lowercase letters on the bars indicate statistical differences for the same group at different times, $P \leq 0.05$, Tukey test.

function. It also caused irritation in the respiratory tract of healthy calves since they manifested greater nasopharynx irritation, with inflammation in the bronchoalveolar region from D3 on. This was characterized by increased cellularity in the region, by an influx of mainly neutrophils, and reduced oxidative metabolism of BA cells. These effects were attenuated by the supplement, which alone stimulated BA IgA production at D7 and increased BA cell oxidative metabolism at D21. In conjunction with the vaccine, the supplement increased oxidative metabolism. It inhibited the increase in BA cellularity without changing its profile on D3 and D7 and stimulated greater production of IgA BA on D21.

Similarly, Gray et al. (2019) also found that an intranasal vaccine containing live attenuated virus caused irritation in the nasopharynx of calves, with infiltration of cells at the site. In their study, proteomics analysis of the region identified increased genes responsible for MHC II expression, neutrophil degranulation, and IL-12 production three days after the vaccine. Although the authors did not explore these results, the local inflammation may have occurred due to the live attenuated viruses contained in the vaccine, which mimic a mild viral infection, causing this local inflammatory response, as suggested by Dou et al. (2015) and Rossi et al. (2021).

This local inflammation can be considered protective if, in addition to providing a greater humoral response, it also increases cellular defense capacity, as cited by Wheat et al. (2020). According to these authors, intranasal administration of an adjuvant, the toll-like receptor agonist liposome 9 (TLR-9), increased the bactericidal capacity and production of nitric oxide (NO) production of CD14 positive cells present in the nasopharynx of calves and stimulated increased production of serum IgG. These responses prevented healthy calves from becoming sick from BRD when housed with sick calves challenged with BRD pathogens. The authors believe that the adjuvant activated the immune system of the upper respiratory region, both cellular and humoral, which suppressed the replication and invasion of pathogens in the region, providing significant protection against the disease.

The most abundant resident cells of the bronchoalveolar region of healthy cattle are the alveolar macrophages, whose main function is phagocytosis and elimination of pathogens via the production of reactive oxygen species (ROS) intracellularly, called oxidative metabolism. This activity can be indirectly measured by the colorimetric assay of the NBT technique. This reagent is phagocytosed by cells such as alveolar macrophages and activated neutrophils and reduced by ERO into a compound called formazan blue (Artner et al. 2018). Thus, reducing this intracellular compound measured in optical density indicates a lower efficiency of BA phagocytes in vaccinated calves, which increases the chance of developing bacterial pneumonia (Bertagnon et al. 2017, McGill & Sacco 2020). In response, there was a significant increase in cellularity in the BA region, with increased neutrophils, especially in D3. Neutrophils are the first defense cells recruited during an inflammatory state (McGill & Sacco 2020).

The increase in antibody production occurred only on D7, which leaves the respiratory tract susceptible to infection, especially on the third day after the vaccine. It is important to remember that the immune response relies on a complex net of elements. In our study, we evaluated only two specific elements: the oxidative metabolite of BA phagocytes and local IgA production. Possibly, the decrease in one of the functions of innate immunity was compensated by an increase in another immune response not evaluated in this research, and that should be the reason why none of the vaccinated animals became ill despite showing clear signs of inflammation in the BA region. They did not show fever, changes in the blood cells count, or auscultation of respiratory noises compatible with pneumonia. It should be emphasized that this experiment was carried out with animals under conditions of a low environmental challenge since they were growing calves, were well-fed, and were in a semi-intensive system. Taube (2020) found that the intranasal vaccine containing live attenuated virus applied in a single dose on the day of entry into the feedlot did not protect cattle against BRD since the vaccinated group had the same incidence of pneumonic lesions and the same slaughter weight as the non-vaccinated animals. For this reason, Chamorro & Palomares (2020) do not recommend intranasally applied live attenuated virus vaccine for feedlot beef cattle.

Although reports on the ability of life-attenuated BRD vaccines to cause disease are still scarce, elimination of the virus from commercial intranasal vaccines has been described for up to 14 days in most vaccinated animals (Walz et al. 2017). Thus, there is concern that attenuated viruses may be able to reverse virulence and infect other animals (Socha et al. 2013, Ollivett et al. 2018).

In order to decrease the inflammatory effects of the vaccine, we studied the effects of probiotics containing *Enterococcus faecium* and *Saccharomyces cerevisiae* on the cellular and humoral response of the respiratory tract. This supplement alone had little effect on the BA region, with increased production of IgA BA on D7 and increased oxidative metabolism of BA cells on D21. Unimpressive results on immunity were also found by Roodposhti & Dabiri (2012) and Flores et al. (2019) when supplementing calves with probiotics. These authors justified that the mantaince of the immunity does not promote expressive immune stimulation because this system is already effective in keeping the animals healthy.

We emphasize that measuring the production of each vaccine virus IgA would be more sensitive for evaluating the humoral response. Ollivett et al. (2018) and Rossi et al. (2021) also measured only the total class of IgA because the increase of these specific titters could increase the total class of immunoglobulins, as our results. That is why the supplement's effects were more expressive after the challenge caused by vaccination. Although the SV group showed higher respiratory tract irritation scores on D3 and D7 than the not-vaccinated group, the increased oxidative metabolism of the BA cells without cellular influx into the region indicates a more effective cellular response than the vaccinated group.

In situations of increased immune challenge, Qadis et al. (2014) and Fomenky et al. (2018) also found that probiotics of similar formulation increased cell communication, resulting in increased numbers and function of T and B leukocytes and blood macrophages from lactating calves with diarrhea or subjected to the stress of weaning. Similarly, Virmond et al. (2020) found that probiotics containing *S. cerevisiae* increased the oxidative metabolism of blood phagocytes of feedlot-finished heifers, which likely accounted for the lower incidence of BRD during feedlot. Mahmound et al. (2020) found increased alveolar macrophage function in neonatal calves supplemented

with *S. cerevisiae* and challenged with BRD, which, along with the greater humoral response, was responsible for less lung inflammation and less disease symptomatology.

Regarding humoral response, both vaccinated groups (V and SV) showed increased BA lymphocyte percentage on D21 and BA IgA production on D7 and D21. Rossi et al. (2021) also found that intranasally administering the vaccine promoted increased IgA BA 7 and 21 days after application. This fact can be explained by the irritation promoted by the vaccine at its application site, which is responsible for more robust immunoglobulin production compared to systemic vaccinations (Ollivett et al. 2018, Gray et al. 2019). Immunoglobulin A is the class of antibodies predominantly produced in mucous membranes, present in the mucus produced by the anterior respiratory tract, whose function is to trap antigens and prevent direct contact of pathogens with the mucosal surface. Although IgA has no bactericidal action, it prevents bacteria from entering the epithelium and neutralizes viruses and bacterial and viral enzymes. Thus, this immunoglobulin is important against viral agents in the respiratory tract (Tizard 2014).

When the supplement was associated with the intranasal vaccine, there was a potentiation of IgA BA production. Similarly, Villot et al. (2020) found that probiotics containing *S. cerevisiae* increased intestinal IgA production in neonatal calves, reducing diarrhea. Although no studies have been found that measure the effects of supplementation on the humoral response in the respiratory tract of calves, it is known that the stimulation of lymphoid tissues associated with mucosa is the same at different mucosal sites, which allows us to extrapolate the data and suggest that the probiotic would also stimulate a greater humoral response in the respiratory tract. This fact was found by Díaz et al. (2018) in murine models when they observed that the *E. faecium*-based probiotic increased the amount and affinity of the humoral response in mice vaccinated against BRD, suggesting that the same effect may also occur in calves.

Considering that there is a recommendation to vaccinate calves in the first week of life against BRD, with vaccines containing the attenuated virus intranasally, new studies are necessary to verify whether this immunostimulant would also potentiate the immune response of immunologically immature calves and whether it is also capable of mitigating the side effects of these vaccines.

CONCLUSION

Supplementation with *Enterococcus faecium* and *Saccharomyces cerevisiae* isolated promoted increased IgA bronchoalveolar (BA) production. In association with the vaccine, it attenuated the inflammation of the respiratory tract produced in response to the vaccine, avoiding the reduction of local innate function, besides potentiating the humoral response of the vaccine containing attenuated virus against bovine respiratory disease (BRD).

Acknowledgments.- Financial support from "Fundação Araucária": Grant 130/2020; support to events, PIBIC scholarship and Capes 001. "Centro de Escrita, Revisão e Tradução Acadêmica" (CERTA - www3.unicentro.br/centrodeescritaacademica), "Universidade Estadual do Centro-Oeste" (UNICENTRO) for assistance with English language translation and developmental editing.

Conflict of interest statement.- The authors declare that there are no conflicts of interest.

REFERENCES

Artner B., Reck A., Coelho A.M., Prediger J.J., Pontarolo D.V. & Bertagnon H.G. 2018. Assessment of the oxidative metabolism of calves' bronchoalveolar cells: comparison between NBT and fluorimetric techniques. Arq. Inst. Biol. 85(1):e0972018. <https://dx.doi.org/10.1590/1808-1657000972018>

Baccili C.C., Martin C.C., Silva K.N., Nichi M., Flores E.F., Vercesi Filho A.E., Pituco E.M. & Gomes V. 2019. Serological response against bovine herpesvirus and bovine viral diarrhea virus induced by commercial vaccines in Holstein heifers. Pesq. Vet. Bras. 39(11):870-878. <https://dx.doi.org/10.1590/1678-5150-PVB-6208>

Batista C.F., Blagitz M.G., Santos B.P., Bertagnon H.G., Parra A.C., Vianna R.S., Lucca G.G., Lima D.M., Santos D.S. & Della Libera A.M. 2012. Maturation of mononuclear phagocytes in the lungs of young calves – in vitro study. J. Dairy Sci. 10(1):5909-5915. <https://dx.doi.org/10.3168/jds.2012-5321> <PMid:22901490>

Bertagnon H.G., Batista C.F., Santos B.P., Lima M.G.B., Bellinazzi J.B. & Della Libera A.M.M.P. 2017. Influence of orchiectomy of seven month old bulls on bronchoalveolar immune function. Arch. Bras. Med. Vet. Zootec. 69(2):310-316. <https://dx.doi.org/10.1590/1678-4162-9214>

Bertagnon H.G., Silva P.E.G., Wachholz L., Leal M.L.R., Fernandes W.R. & Benesi F.J. 2007. Imunoglobulinas no trato respiratório de bezerros sadios durante o primeiro mês de vida. Pesq. Vet. Bras. 27(12):487-490. <https://dx.doi.org/10.1590/S0100-736X2007001200003>

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? Vet. Clin. N. Am., Food Anim. Pract. 36(2):461-472. <https://dx.doi.org/10.1016/j.cvfa.2020.03.006> <PMid:32451035>

Díaz A.M., Almozni B., Molina M.A., Sparo M.D., Manghi M.A., Canellada A.M. & Castro M.S. 2018. Potentiation of the humoral immune response elicited by a commercial vaccine against bovine respiratory disease by *Enterococcus faecalis* CECT7121. Benef. Microbes 9(4):553-562. <https://dx.doi.org/10.3920/BM2017.0081> <PMid:29633631>

Dou Y., Fu B., Sun R., Li W., Hu W., Tian Z. & Wei H. 2015. Influenza vaccine induces intracellular immune memory of human NK cells. PLoS One 10(3):e0121258. <https://dx.doi.org/10.1371/journal.pone.0121258> <PMid:25781472>

Flores G.V.B., Thomaz G.R., Horner Netto W., Rossi P.S., Strickler F., Bertagnon H.G., Seki M.C. & Carrasco A.O.T. 2019. Effect of *Enterococcus faecium* and *Saccharomyces cerevisiae* on immune response, hematological parameters and weight gain of calves fed corn silage. Vet. Anim. Sci. 26:1-11. <https://dx.doi.org/10.35172/rvz.2019.v26.353>

Fomenky B.E., Chiquette J., Lessard M., Bissonnette N., Talbot G., Chouinard Y.P. & Ibeagha-Awemu E.M. 2018. *Saccharomyces cerevisiae* var. *boulardii* CNCM I-1079 and *Lactobacillus acidophilus* BT1386 influence innate immune response and serum levels of acute-phase proteins during weaning in Holstein calves. Can. J. Anim. Sci. 98(3):576-588. <https://dx.doi.org/10.1139/cjas-2017-0120>

Fulton R.W. 2009. Bovine respiratory disease research (1983-2009). Anim. Health Res. Rev. 10(2):131-139. <https://dx.doi.org/10.1017/S146625230999017X> <PMid:20003649>

Fulton R.W. 2015. Impact of species and subgenotypes of bovine viral diarrhea virus on control by vaccination. Anim. Health Res. Rev. 16(1):40-54. <https://dx.doi.org/10.1017/S1466252315000079> <PMid:26050571>

Gray 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. J. Proteom. 204(1):103397. <https://dx.doi.org/10.1016/j.jprot.2019.103397> <PMid:31146050>

Mahmound 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. J. Anim. Sci. 98(8):1-16. <https://dx.doi.org/10.1093/jas/skaa252> <PMid:32780814>

McGill J.L. & Sacco R.E. 2020. The immunology of bovine respiratory disease: Recent advances. Vet. Clin. N. Am., Food Anim. Pract. 36(2):333-348. <https://dx.doi.org/10.1016/j.cvfa.2020.03.002> <PMid:32327252>

Ollivett T.L., Leslie K.E., Duffield T.F., Nydam D.V., Hewson J., Caswell J., Dunn P. & 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. J. Dairy Sci. 101(1):8159-8168. <https://dx.doi.org/10.3168/jds.2017-14271> <PMid:29908805>

Qadis A.Q., Goya S., Yatsu M., Kimura A., Ichijo T. & Sato S. 2014. Immune-stimulatory effects of a bacteria-based probiotic on peripheral leukocyte subpopulations and cytokine mRNA expression levels in scouring Holstein calves. J. Vet. Med. Sci. 76(5):677-684. <https://dx.doi.org/10.1292/jvms.13-0534> <PMid:24451928>

Roodposhti P.M. & Dabiri N. 2012. Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. Asian-Australasian J. Anim. Sci. 25(9):1255-1261. <https://dx.doi.org/10.5713/ajas.2011.11312> <PMid:25049688>

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. Vet. Q. 41(1):97-106. <https://dx.doi.org/10.1080/01652176.2020.1870019> <PMid:33375918>

Socha W., Rola J., Bednarek D., Urban-Chmiel R. & Żmudziński J.F. 2013. Shedding course of bovine respiratory syncytial virus and bovine parainfluenza 3 virus in calves vaccinated intranasally. Bull. Vet. Inst. Pulawy 57(4):479-483. <https://dx.doi.org/10.2478/bvip-2013-0083>

Taube M.J. 2020. Comparação de vacinas comerciais intranasal e intramuscular contra viroses respiratórias em bovinos confinados. Dissertação de Mestrado, Universidade Estadual do Centro-Oeste (UNICENTRO-PR), Guarapuava, PR. 76p.

Tizard I.R. 2014. Imunologia Veterinária. 9ª ed. Elsevier, Rio de Janeiro. 568p.

Villot C., Chen Y., Pedgerachny K., Chaucheyras-Durand F., Chevaux E., Skidmore A., Guan L.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. J. Dairy Sci. 103(9):8615-8628. <https://dx.doi.org/10.3168/jds.2020-18274> <PMid:32684462>

Virmond M.P., Rossi P.S., Antunes A.V., Mattei R.I., Schllemer N.R., Thomaz G.R., Garbossa G., Neumann M. & Bertagnon H.G. 2020. Fresh yeast additives improve immune parameters and reduce respiratory disease in heifers finished in feedlots. Semina, Ciênc. Agrárias 41(6Supl.2):3177-3188.

Walz P.H., Newcomer B.W., Riddell K.P., Scruggs D.W. & Cortese V.S. 2017. Virus detection by PCR following vaccination of naive calves with intranasal or injectable multivalent modified-live viral vaccines. J. Vet. Diagn. Investig. 29(5):628-635. <https://dx.doi.org/10.1177/1040638717709039> <PMid:28545321>

Wheat W., Chow L., Rozo V., Herman J., Still Brooks K., Colbath A., Hunter R. & Dow S. 2020. Non-specific protection from respiratory tract infections in cattle generated by intranasal administration of an innate immune stimulant Non-specific protection from respiratory tract infections in cattle generated by intranasal administration of an innate immune stimulant. Plos One 15(6):e0235422. <https://dx.doi.org/10.1371/journal.pone.0235422> <PMid:32584899>