ORIGINAL ARTICLE

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Intranasal and intramuscular commercial vaccines against respiratory viruses in steers finished in feedlot

Vacinas comerciais intranasal e comercial contra viroses respiratórias em garrotes terminados em confinamento

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ABSTRACT: Bovine respiratory disease (BRD) is a condition of high incidence, especially in feedlot cattle, however, there is still no consensus on the most effective vaccine protocol for its control. The objective was to evaluate two vaccine protocols against BRD in steers finished in feedlot and their influence on respiratory indicators and weight gains. This study included 42 ½ Angus blood steers, distributed in control, IN, and IM groups. On the IN protocol, the steers received a single vaccine dose on the day of the feedlot entrance. For the IM protocol, they received two vaccine doses, the primo vaccination was performed 21 days prior to the feedlot and the reinforcement on the day of feedlot entrance. BRD indicators, body weight gain, and serum levels of immunoglobulin-G, immunoglobulin-A, and haptoglobin were measured. At slaughter, the presence and severity of pneumonia were analyzed. IM protocol presented a lower BRD indicators and incidence of pneumonia (P=0.0004 CO and P=0.03, IN) and better performance (body weight gain and slaughter weight (P=0.05) than the other groups. IN presented more respiratory disease indicators from on D7 and the same performance of the control group. We conclude the IM protocol was more effective for steers finished in feedlot.

KEYWORDS: Confined; Immunity; pneumonia; beef cattle; prophylaxis.

RESUMO: Doença respiratória de bovinos (DRB) é uma condição de alta incidência, especialmente em bovinos confinados, e ainda não há um consenso sobre qual protocolo vacinal é mais efetivo. Desta maneira o objetivo foi avaliar a eficiência de dois protocolos vacinas contra DRB em garrotes terminados em confinamento em relação aos indicadores de doença respiratória e ganho de peso. Estudou-se 42 garrotes ½ sangue Angus, distribuídos nos grupos: Controle, Intranasal (IN) e Intramuscular (IM). O protocolo IN foi realizado com uma dose única da vacina no dia da entrada no confinamento. O protocolo IM foi realizado com duas does da vacina, sendo a primovacinação realizada 21 dias antes da entrada no confinamento e o reforço realizado na entrada do confinamento. Indicadores de DRB, ganho de peso, haptoglobina sérica e imunoglobulinas séricas A e G foram mensuradas. Ao abate, analisou-se a presença e severidade de pneumonia. O IM apresentou menores indicadores de doença respiratória e ocorrência de pneumonia (P=0.0004 CO e P=0.03, IN) e melhor desempenho (maior ganho de peso e maior peso ao abate P=0.05) que os demais grupos. IN apresentou mais indicadores de doenças respiratórias a partir do D7 e a mesmo desempenho que o grupo controle. Conclui-se que o protocolo IM foi o mais efetivo para garrotes terminados em confinamento.

PALAVRAS CHAVES: Confinado, imunidade, pneumonia, bovino de corte, profilaxia

INTRODUCTION

The bovine respiratory disease (BRD) is a disease of high incidence worldwide, particularly in feedlot cattle, which has motivated the development of protocols for its control. However, there is still no consensus about which would be the most effective (Chamorro; Palomares, 2020; Taylor *et al.*, 2010). The most used protocols are metaphylaxis or vaccination practices, in which the use of commercial vaccines presents advantages over the former because it is less expensive, does not leave residues in the carcass, and does not cause bacterial resistance. However, it is recommended that it be performed in two doses before the stressful event, which can be an obstacle to the productive system of finishing cattle (Chamorro; Palomares, 2020; Taylor *et al.*, 2010).

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There are several vaccine formulations in the market, and most of them are directed against the main viral agents of BRD: bovine herpesvirus (BHoV-1 and 2), bovine viral diarrhea virus (BVDV), bovine parainfluenza virus (PI-3) and bovine syncytial virus (BRSV), indicated for parenteral administration in two doses (Chamorro; Palomares, 2020; Lorenz *et al.*, 2011). As for the intranasal route, the commercial vaccines normally do not contain BVDV in their formulation, and present advantages in relation to the parenteral ones for stimulating a greater local immune response (Grey *et al.*, 2019; Rossi *et al.*, 2021), besides being indicated as a single dose, on the day the cattle enters the feedlot (Chamorro; Palomares, 2020).

Facing the existing commercial vaccines against BRD it is noteworthy that several factors may interfere in the effectiveness of vaccine protocols, starting by the route of application, number of vaccine doses, time of vaccination and environmental challenge experienced by the animals (Chamorro; Palomares, 2020; Magalhães *et al.*, 2017).

Considering that a greater handling of beef cattle induces more stress on the animals, the doubt remains about what would be the best alternative vaccine protocol for commercial feedlots. Thus, a parenteral vaccine, to be applied in two doses before entering the feedlot, and an intranasal vaccine, to be applied in a single dose, on the day of the feedlot entry, were selected to verify which protocol would be the most adequate for beef cattle finished in feedlots, taking into account the occurrence of respiratory alterations and its influence on weight gain.

MATERIAL AND METHODS

This research was approved by the animal use ethics committee CEUA/UNICENTRO 008/2018).

Experimental design

The study was carried out in a commercial feedlot in the city of Guarapuava, Paraná, in the south-central region of the state of Paraná, with geographical coordinates of 25° 23' 26" South latitude and 51° 27' 15" West Greenwich. The region has an altitude of approximately 1100 m and a moderate subtropical climate, with annual rainfall of 1.944 mm, minimum and maximum temperatures of 12.7 °C to 23.5 °C, and relative humidity of 77.9%. The experiment was conducted in the period from June to November. During this period, the maximum ambient temperature was 22 °C and the minimum was 11.6 °C.

The feedlot had a capacity for 425 cattle to be arranged in 25 concrete stalls of 50m², arranged side by side, allowing partial contact of the animals between the stalls. The stalls had good ventilation, and were cleaned by scraping the floor every other day. Each stall had a concrete feeder an automatic concrete waterer, and they share the same water.

From this feedlot, 42 half-blood Angus calves, aged 9 (±1) months and weighting 755, 7 ±4 lb (345 ± 4 kg) were included in a randomized clinical trial in 3 distinct groups, with 14 animals each, which were distributed in the three experimental stalls with heterogeneity of treatments. The animals for each treatment had a homogeneity of height, weight and body score condition (BSC), followed from the time of allocation to the stalls until slaughter, at 87 days.

The control group (CO, n=14), did not receive a vaccine. The intranasal group (IN, n=14) received a single dose of the commercial vaccine composed of BoHV-1, PI-3 and BRSV viruses (attenuated samples) (Inforce 3®, Zoetis, São Paulo, Brazil), administered 1 mL in each nostril. The animals were contained in a hydraulic trunk, with an nose ring to elevate the head, which was kept elevated for 1 minute, on the day of entry into the feedlot. And the intramuscular group (IM, n=14) was vaccinated with two doses of 2 mL each, with an interval of 21 days, of the commercial vaccine composed of attenuated samples of BoHV-1, PI-3, BRSV and BVDV viruses (inactivated strains 5960 cytopathic and 6309 non-cytopathic) (Cattle Master 4®, Zoetis, São Paulo, Brazil), with the first dose at 21 days before the animals entered the feedlot. At the same time, all animals were weighed, but not equally restrained, to verify whether the very handling of the animals during vaccine administration could also interfere in the occurrence of BRD indicators and weight gain.

The steers were originally raised on oat and ryegrass pasture, and when they reached approximately 345 kg BW, they were confined in the stall. They were gradually adapted during 21 days for a diet consisting of 5kg commercial feed (Agramulti Bovinos 20®, Agrária, Guarapuava, Brazil) and 15 kg of corn silage per animal. During the animal diet adaptation, 0.5 kg of concentrate were added every 2 days until totaling 5 kg (table 1). The food was provided twice a day, at 6:00 am and at 5:30 pm, in the form of a total mixed ration (TMR). To adjust the volumes of feed provided, leftover food was weighed daily in the trough, and the food was adjusted so that 5% of the dry matter was left.

Exclusion factor

Animals with non-respiratory changes were excluded from the experiment (two animals with lameness and two animals with anaplasmosis). Animals that did not reach the body weight for slaughter at 87 days of feedlot were excluded from the pulmonary analyses. Thus, the experiment was conducted with 11 animals in the IN, 13 animals from CO and 14 animals in IM for the analyses of body weight and respiratory disease indicators and nine animals in the IN group, 13 animals in the CO group and 14 animals in the IM group for lung analyses

Table 1. Chemical composition of feed used in animal feed and average values of the experimental diet, based on total dry matter (DM)

Parameter	Corn Silage	Concentrate	Experimental Diet[1]
Dry matter (DM) %	33,83	90,40	62,12
Mineral matter (MM) % -DM	2,51	6,36	4,44
Crude Protein (CP), %- DM	8,44	20,20	14,32
Ethereal Extract (EE), %-DM	2,65	2,05	2,35
Neutral Detergent Fiber (NDF), % DM	46,14	31,47	38,80
Acid Detergent Fiber (ADF), % 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; e Zn: 95,20 mg

Sample analyses

In the beginning (D0-D8 and D10- D18), middle (D32-D44), and end (D71-79) of the feedlot, the presence of mucopurulent nasal secretion (evaluated by visual inspection of the nostrils), measurement of the orbital temperature (infrared thermometer digital, positioned 10 cm from the center of the eyeball at the time of weighing the animals, were evaluated at the same time of day on each of eight consecutive days between each experimental timepoint. The presence (score 2) or absence (score 1) of nasal secretions for each sample timepoint were calculated. From these medians, the frequency of animals with mucopurulent nasal discharge was identified. The means of orbital temperature for each sample timepoint were calculated.

The weigh body of the animals (digital scales, without prior fasting) were measured, always at the same time of day, on days 0, 7, 44 and 79.

On days 0, 15, 44 and 79,10 mL of blood was collected by puncture of the coccygeal vein in plastic tubes without anticoagulant. The blood was centrifuged (3000 rpm x 15 min), and the serum was frozen at - 4°C until the end of experiment. Haptoglobin, IgA and IgG and BVDV antibodies were measurement by ELISA technique, as recommended by the manufacturer (cat EB0011, Bovine HP haptoglobin Elisa Kit®, FineTest, Wuhan, China; cat: ab 190516 IgA Cow ELISA Kit®, Abcam, Cambridge, United States and cat ab190517, Bovine IgG ELISA Kit®, Abcam, Cambridge, United States; BVBV total ab test, Idexx, São Paulo, Brazil).

At 87 days the animals were slaughtered and a macroscopic pulmonary examination was performed to identify presence and severity of lung consolidation, according to Garbossa et al. (2023) Absence of lesion- score 1; score 2 for lesions up to 50%, score 3- lesions between that 50% to 75% and score 4, lesions greater than 75% of the ventral cranial lobe. Fragments of the ventral cranial lobe, in the transition area between normal tissue and areas of consolidation, of approximately 2 cm^2 were then harvested for histopathological evaluation. The tissues were fixed in 10% formalin for 48 hours and embedded in paraffin for histopathological slides, which were stained with hematoxylin and eosin (HE), and observed under light microscopy (40X). The fragments were classified as absence of pneumonia, purulent catarrhal pneumonia, fibrinous, interstitial or granulomatous, according to Ceribasi et al. (2014).

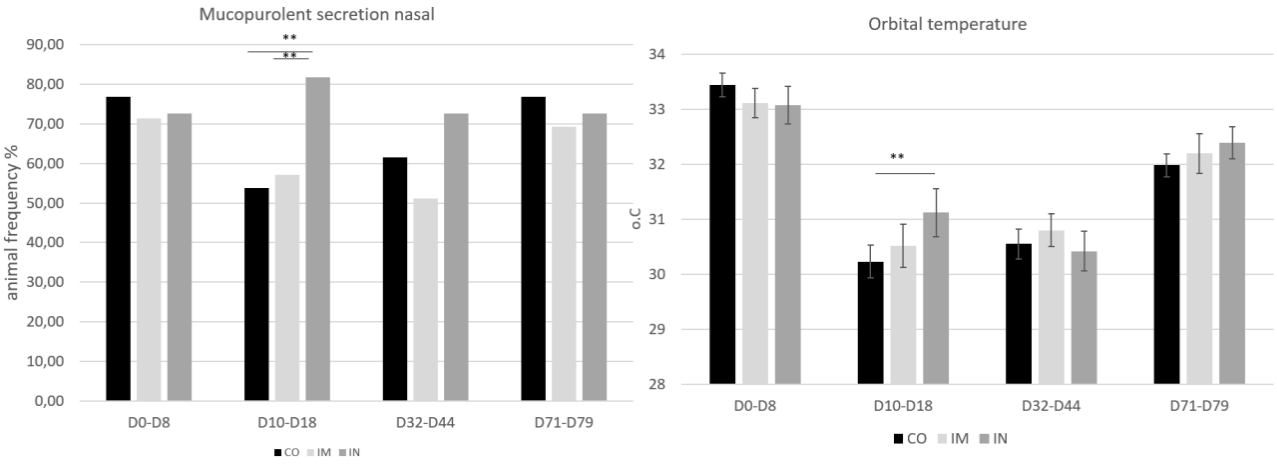
Statistical Analysis

The data were submitted to statistical analysis using Instat Graphpad® statistical software. For evaluation of immunoglobulins, haptoglobin, orbital temperature and weight gain data, parametric tests of repeated measures Anova and Tukey's test as post-test were performed. The frequency of animals with presence of mucopurulent nasal secretion and with pneumonia and their respective scores were subjected to nonparametric Chi Square test. Data were considered with statistical significance when $P \leq 0.05$ and statistical trend when $P > 0.05$ and $P \leq 0.1$.

RESULTS

At D0, all animals had positive serology for BVDV, which remained positive throughout the experiment for all animals. As for the indicators of respiratory disease, it was noted that more animals in the IN group showed mucopurulent nasal secretion and orbital temperature (figure 1) than the control group (P=0.01 and P=0.0004 respectively) and more mucopurulent nasal secretion than the IM (P=0.09) at D10-18. At the other times there was no treatments effect.

The body average gain (DAG) and the body weight (BW) of animals are in table 2. The IN and CO gain less weight daily than the IM group that between D44 to D79 and between D79 and D87 (P=0.05). The IN had a lower slaughter body weight and carcass weight than IM (P=0.05), but the same of the CO. The IM had the same slaughter body weight and carcass weight than CO.



IM: intramuscular vaccine group (14 animals), IN: intranasal vaccine group (11 animals), CO: Control group (13 animal), non-vaccinated. *indicate significant difference for treatment P≤0.05, **P≤0.001 and *** P≤0.0001

Figure 1. Respiratory diseases indicator of feedlot steers according to the vaccine protocol used.

Table 2. Body weight and carcass yield (%) of feedlot steers according to the vaccine protocol used.

		Daily average weight gain (kg day^{-1})				Body Weight (Kg)			
		D0-D8	D10-D18	D32- D44	D71-87	D0	D87	Carcass weight	Carcass yield (%)
IM	Mean	2.20a	1.86a	1.81a	1.84a	347.93a	510.86a	274.80a	53.08a
	SEM	0.31	0.08	0.07	0.08	5.19	7.88	7.04	3.50
IN	Mean	2.04a	1.60a	1.56b	1.49b	347.50a	477.54b	255.14b	53,43a
	SEM	0,37	0,16	0.10	0.18	4.66	10.06	8.71	1.55
CO	Mean	2.36a	1.82a	1.65b	1.70b	345.63a	491.86ab	265.60ab	54.00a
	SEM	0.22	0.07	0.06	0.14	3.89	8.55	7.53	2.10
	Pvalue	0.77	0.24	0.05	0.05	0.4	0.05	0.05	0.66

IM: intramuscular vaccine group (14 animals), IN: intranasal vaccine group (11 animals), CO: Control group (13 animal), non-vaccinated. SEM: Standard error of the mean. Differents letters in the same columns indicate significant difference for treatment interaction.

The serum IgG and IgA levels are shown in figures 2. The CO started with higher serum IgG levels than the other groups (P=0.01 and 0.05), and lower serum IgA than IM. Both serum immunoglobulins remained stable over time. There was an increase in serum IgG in the IM Group on D7 and D44 compared with D0 (P=0.03), while serum IgA did not change over time (P=0.12). There was a decrease in serum IgA at all times compared to D0 (P=0.0001) in the IN group. The serum IgG always was lower than CO or IM and serum IgA was lower than IM group on D 79 (P=0.05).

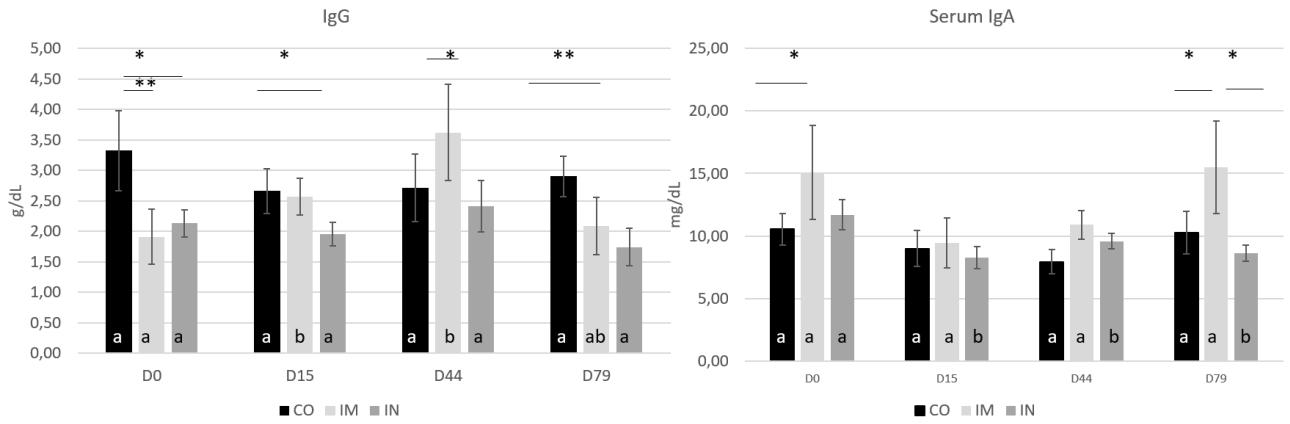
The serum haptoglobin is in figure 3. The IM group had higher serum haptoglobin levels than the other groups at D0 and D15. Later these levels decreased on D44 and D79 (P=0.03) in the IM group. The IN had higher serum haptoglobin levels than CO (P=0.001) on D7. And these levels were higher than CO and IM on D44 and D79 groups (P=0.003 and 0.04). The serum haptoglobin level remained stable in CO.

The macroscopic lesions were characterized by reddish purple consolidation and lobes with focal points with atelectasis. There were no pleural changes or adhesions between the lung lobes. No animal had lesion score 4. IM had more animals without macroscopic lung changes (score 1) than the CO group (P=0.0005) and the IN (P=0.05). The IN had a greater number of animals without lung injury than the CO, without statistical difference (P=0.24) (Figure 4).

IM had lower incidence of inflammatory infiltrated in the lung than CO (P=0.0004) and IN (P=0.03), IN had the same incidence of inflammatory infiltrated in the lung than CO (P=0.40) (figure 4). The types of pneumonia had equal distribution between the groups, and were mainly characterized by interstitial pneumonias, mainly presenting lesions such as thickening of the interalveolar septum, degeneration or desquamation of the bronchiolar epithelium, and intra-alveolar and peribronchiolar mononuclear infiltrate. Neither purulent nor granulomatous catarrhal pneumonias were found.

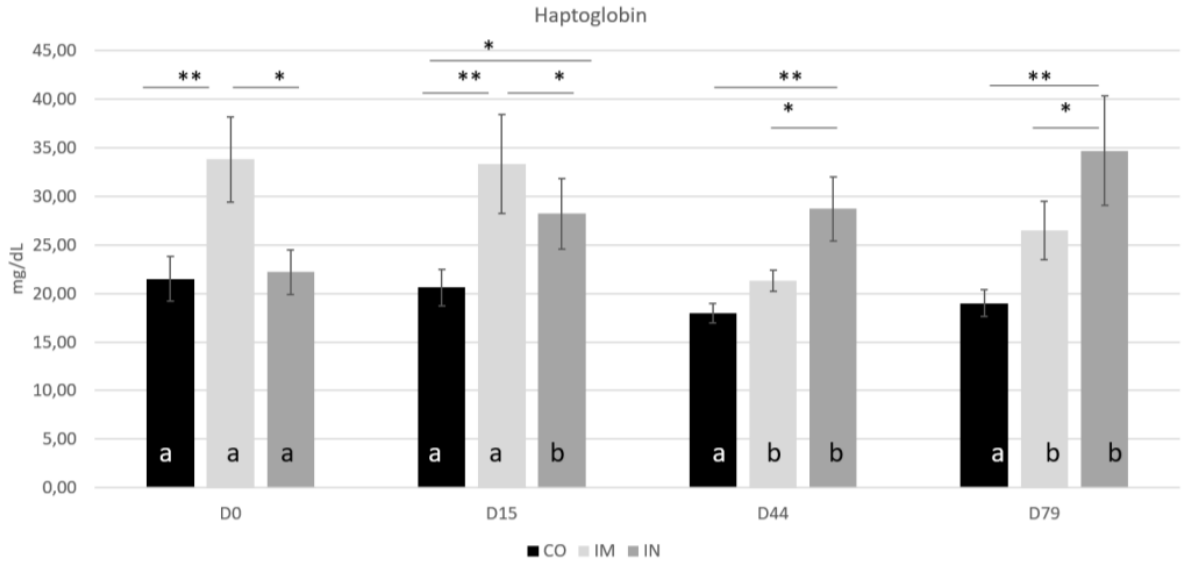
DISCUSSION

We observed that the two-dose intramuscular vaccine protocol administered 21 days prior to the feedlot was more effective in this feedlot when evaluating the BRD indicators,



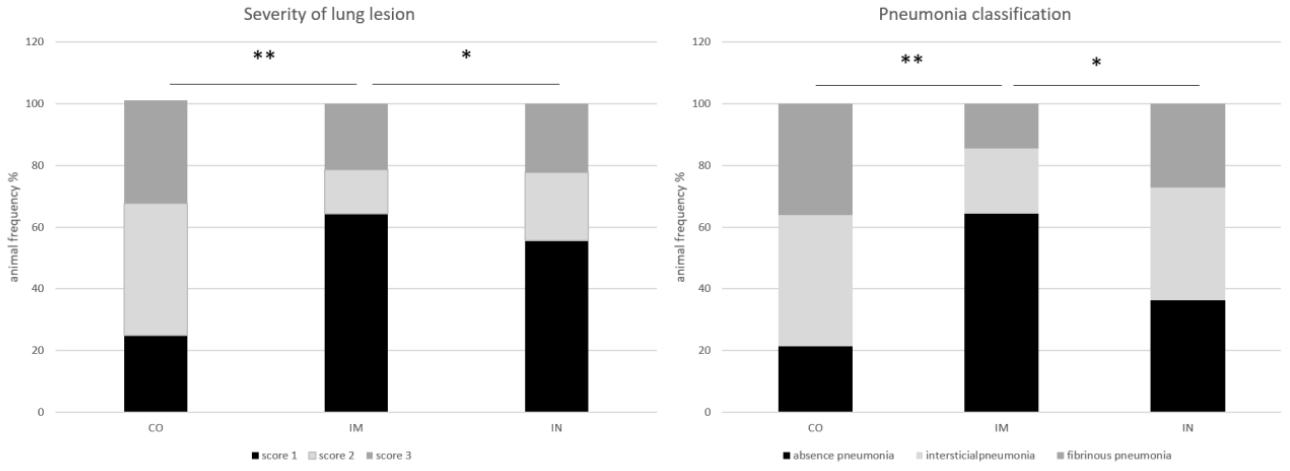
Differents letters in the same bars indicate significant difference for time interaction in each group P≤0.05 *indicate significant difference for treatment P≤0.05, **P≤0.001 and *** P≤0.0001.

Figure 2. Serum IgG and IgA of feedlot steers according to the vaccine protocol used



Differents letters in the same bars indicate significant difference for time interaction in each group P≤0.05 *indicate significant difference for treatment P≤0.05, **P≤0.001 and *** P≤0.0001 .

Figure 3. Serum haptoglobin of feedlot steers according to the vaccine protocol used.



IM: intramuscular vaccine group (14 animals), IN: intranasal vaccine group (11 animals), CO: Control group (13 animal), non-vaccinated. Score 1: Absence of injury, score 2 - injuries up to 50% of the pulmonary cranioventral lobe, Score 3, injuries between 50% to 75% of the pulmonary cranioventral lobe. Differents letters in the same bars indicate significant difference for time interaction in each group P≤0.05 *indicate significant difference for treatment P≤0.05, **P≤0.001 and *** P≤0.0001, Chi-square test.

Figure 4. Animal frequency of pneumonia and their severity in feedlot steers according to the vaccine protocol used.

immunoglobulin secretion and body weight than the single-dose intranasal vaccine protocol at the feedlot entrance, which did not show benefits in this feedlot.

The both vaccines used contain multivalent modified-live virus such: BoHV-1, PI-3 and BRSV, but only the IM vaccine presents in its composition inactivated BVDV. Possibly it is one of the reasons responsible for the lower control of BRD in this feedlot by IN protocol, considering that at D0, all animals already had antibodies against the agent, so there was a virus circulation in the herd.

The IM had higher serum haptoglobin levels than the subsequent times and the other groups. The serum haptoglobin is an effective inflammatory biomarker in bovine species (Wolfger *et al.*, 2015). Their increase could indicate a viremia due by a vaccine protocol, which has already been reported by Fulton *et al.* (2003) in cattle vaccinated intramuscularly with attenuated BVDV. Another possible hypothesis would be the IM vaccine protocol caused a greater stress in the animals, because the handling to apply the two doses of vaccine in the animals. This stress promoted decrease of the lymphocytes activity and antibodies production and stimulating the secretion of serum haptoglobin (Blecha, 2000; Sporer *et al.*, 2008), which would explain the lower serum IgG levels and higher serum haptoglobin levels in the IM and IN groups in the beginning of feedlot. From D44 on, there was a reduction in stress, which restored serum haptoglobin levels, and allowed the increasement of serum IgG levels and lately in the serum IgA in the IM group.

In the IN group, the serum IgG also was lower than CO at D0 and D15, it is believed that they were caused by the stress of the vaccine application, placement of the ant and keeping the head of the animal elevated. This handling did not occur in the other groups (Sporer *et al.*, 2008).

At D10-D18, there were an increase of orbital temperature, serum haptoglobin and animals with mucopurulent nasal secretion and a decrease of serum IgA. These findings indicate this vaccine route promoted a greater irritation in the respiratory tract, similarly to what was found by Grey *et al.* (2019) and Rossi *et al.* (2021) during the establishment of vaccine immunity. Both authors inferred that this route of vaccination depresses pulmonary cellular immune response, and this immunosuppression can be worsen by stress caused by handling of animal during the vaccine applications. For this reason, Chamorro; Palomares (2020) have doubts about the use of vaccine containing attenuated virus for intranasal application for beef cattle.

From D15 on, the serum haptoglobin levels remains higher and serum IgA remains lower than D0. As this immunoglobulin is responsible for mucosal protection and has as its main function the apprehension of antigens in mucus, preventing their contact with the respiratory tract (Chase, 2022), it is believed that the greater occurrence of BRD at these timepoints in the IN caused the serum translocation of IgA to the respiratory region to neutralize pathogens, which has already been reported by Woolums *et al.* (2013).

From D44, the IN group also had lower daily average weight gain than the IM, which result in a lower final body weight and slaughter weight. This protocol did not prevent BRD, as evidenced at slaughter, when this group had a higher frequency of animals with pneumonia than IM, and the same frequency of the CO.

Such finding is in agreement with Rezac *et al.* (2014) and Tennant *et al.* (2014), who reported that the main reason for reduced performance in feedlot cattle is BRD, which even in its subclinical form, observed only at the time of slaughter, already impacts productive performance.

Thus it was noted that the IN protocol did not significantly prevent the occurrence of BRD, nor the drop in performance of cattle finished in feedlot, due to some factors. As already mentioned, the vaccines used had very similar composition, but the IN did not contain BVDV, a virus present in this feedlot and in many cattle feedlots in the world whose vaccine protection is questionable due to the high mutation capacity of the virus (Smith, Step; Woolums *et al.*, 2022).

Furthermore, two vaccine doses by parenteral route induce higher serum antibodies production than only one dose (Chamorro; Palomares, 2020), but one dose of intranasal vaccine is still more effective than not vaccinating (Chamorro; Palomares, 2020; Ollivett *et al.*, 2018). These results were contrary to the results of the present research, where not vaccinating was similar to the IN vaccine protocol possibly due to immunosuppression caused by the stress of vaccination or also due to the environmental challenge faced by the animals.

The adoption of two vaccine doses intranasally is feasible and could result in greater protection of animals against BRD, however it would affect one of the main advantages of the adopted IN protocol compared to the IM protocol, in relation to reducing the labor and stress of containing the animals twice for vaccination, and the cost per protocol, considering that the IN vaccine costs $1.77/animal and the IM costs $2.95/animal.

Finally, one of the obstacles for the establishment of vaccine protocol for cattle finished in feedlots is the date of vaccination. According to Chase (2022), the ideal would be to vaccinate between 60 and 15 days before the feedlot entry, but the producer does not always have access to the animals before confinement, because normally these animals are acquired from another location. Therefore, the IN protocol is indicated as a single dose on the day of entry into the feedlot, since it stimulates a greater production of antibodies than the parenteral route (Chamorro; Palomares, 2020; Magalhães et al., 2017), unfortunately the results of the present study showed that for

this feedlot situation, the IN protocol did not prevent BRD nor its interference on weight gain.

CONCLUSION

It was concluded that the IM vaccine protocol minimized the occurrence of BRD, and guaranteed higher weight gain of cattle finished in commercial feedlot in relation to the IN protocol and the non-vaccination. The IN protocol did not influence the occurrence of pneumonia or the body weight of the steers at the time of slaughter.

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