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Use of Restricted Nursing and a Bovine Appeasing Substance on the Welfare, Growth Performance, and Response to Vaccination of Beef Calves at Weaning

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ABSTRACT

This study evaluated the effects of restricted nursing (RN) and the administration of a bovine appeasing substance (BAS) given 8 days before (d-8) and on the day of weaning (d0) as a strategy to mitigate stress and optimize the post-weaning growth performance of beef calves, with emphasis on behavior and response to vaccination. Forty male Nelore calves were randomly assigned to four treatments with ten replicates: control (CON)—topical administration of 5 mL of saline solution on d0; BAS-8—topical administration of 5 mL of BAS on d-8; BAS0—topical administration of 5 mL of BAS on d0; and RN—calves allowed to nurse only one hour/day from d-8 to d0. Calves in the CON treatment group showed lower average daily gain compared to the other treatments ($p < 0.05$). There was a trend ($p = 0.09$) for BAS-8 and BAS0 to result in lower temperament scores when calves entered the restraint chute. BAS0 calves had greater ($p = 0.05$) serum titer concentrations against rabies compared to the other treatments. The RN and BAS administration eight days before and on the day of weaning was effective in improving growth performance in beef calves.

1 | Introduction

Weaning is an event that has been widely studied due to its negative effects on animal welfare and its direct influence on the growth performance of beef cattle. It is considered one of the main stressors in calf management, especially when carried out abruptly, a practice known as conventional weaning [1, 2].

Conventional weaning, which involves the permanent separation of cows and calves at approximately seven months of age, leads to changes in stress-related hormonal mediators, animal behavior, immune function, and consequently affects post-weaning growth [3, 4]. Therefore, information on management alternatives that can minimize the impact of cow–calf separation at weaning is essential to ensure improvements in calf performance during the first weeks after weaning, a critical period of adaptation to a new phase of life. Several alternative weaning approaches have been explored to reduce stress during this transition, including temporary restriction to nursing (RN) [5] and administering bovine appeasing substance (BAS) [6].

The RN is a pre-weaning management tool that involves the gradual separation of cows and calves, allowing them to have only one daily contact [5]. This practice aims to prepare the calf for permanent maternal separation, potentially reducing stress and ensuring post-weaning growth [2, 7]. Gradual weaning is a

common practice in some dairy cattle production systems as described in a recent review of Welk et al. [8]; however, there are few reports of its use in beef production systems. In an experiment by Lay et al. [5], RN of Brahman calves at earlier life reduced stress around weaning. However, we hypothesized that RN not in early life but in the week before weaning would reduce weaning stress and improve growth performance, behavior, and response to vaccination of beef calves.

BAS is a pheromone composed of a mixture of fatty acids, with a structure similar to the appeasing substance naturally secreted by the sebaceous glands of the cow's mammary gland [1, 9]. Upon reaching the olfactory epithelium of calves, this substance activates the neuroendocrine system, reducing stress and inducing a sense of security, which makes the animal feel less threatened [9].

The BAS administration in nuchal skin area of calves is a tool with the potential to reduce stress in cattle, particularly during weaning and feedlot entry. Several studies [1, 10–13] have shown that BAS administration at weaning improves chute temperament and weight gain, and enhances response to vaccination. However, information on BAS administration before weaning remains scarce in the literature. BAS typically affects the animal for up to 15 days [12]. Therefore, its administration in the week before weaning may help significantly reduce stress during this stage, contributing to improved performance in subsequent phases.

Thus, this study aimed to evaluate the effects of RN and a BAS administration (eight days before and on the day of weaning) as a strategy to mitigate stress and optimize the post-weaning growth performance of beef calves, evaluating behavior and response to vaccination.

2 | Materials and Methods

2.1 | Location and Procedures

The experiment was conducted at the Beef Cattle Farm of the Mato Grosso do Sul State University, Aquidauana University Unit (UEMS/UUA), located in the municipality of Aquidauana, Mato Grosso do Sul, Brazil (20º27′ S and 55º40′ W). According to the Köppen classification, the climate of the study region is classified as Tropical Savanna, characterized by a dry winter and summer temperatures exceeding 35°C. The study was approved by the Animal Ethics Committee (CEUA) of UEMS under protocol number 007/2023.

2.2 | Animals and Treatments

A total of 40 male Nelore beef calves were used in this experiment. At the beginning of the study, the animals had an average age of 210 days (± 10) and an average body weight (BW) of 220 kg (± 34). The experiment lasted a total of 106 days, covering the eight days before weaning, the weaning day, and the subsequent 97 days corresponding to the post-weaning period (Figure 1).

The animals were kept in a continuous grazing system on *Urochloa decumbens* pastures, with free access to a mineral supplement (ProBeef 800; Nutron Animal Nutrition; with a prescribed intake of 25–35 g/100 kg of BW) and water *ad libitum*. The calves were stratified according to BW, and then randomly assigned to four treatments with ten replicates: control (CON)—topical administration of 5 mL of saline solution (0.9% NaCl) on d0; BAS-8—topical administration of 5 mL of BAS on d-8; BAS0—topical administration of 5 mL of BAS on d0; and RN—restricted nursing, where the calves were grouped with their mothers only between 16:30 and 17:30 h for one week, from d-8 to d0. The CON and BAS solutions (Secure Cattle, IRSEA Group, Quartier Salignan, France) were topically applied to the nuchal skin area of each calf.

Animals were maintained as a single group on the same pasture throughout the experimental period, with the following exceptions: calves in the RN group were separated from the main group from d-8 to 0 to facilitate nursing procedures; calves in the BAS-8 and BAS0 groups were kept in separate groups from d-8 to 7 and d0 to 15, respectively, to prevent potential crossover effects, as the estimated duration of BAS persists for up to 15 days following administration, according to the manufacturer.

At d0, a vaccine containing a Pasteur-inactivated rabies virus adsorbed on aluminum hydroxide gel and produced using cell cultures (Raivacel Multi, MSD Saude Animal, Montes Claros, MG, Brazil) was subcutaneously applied to all calves at a dose of 2 mL/animal.

2.3 | Growth Performance

For the evaluation of growth performance, the calves were weighed using a digital electronic scale at the beginning of the experimental period (d-8) and on different days after weaning (d0, d12, d36, d67, and d97). From the weighings, BW and average

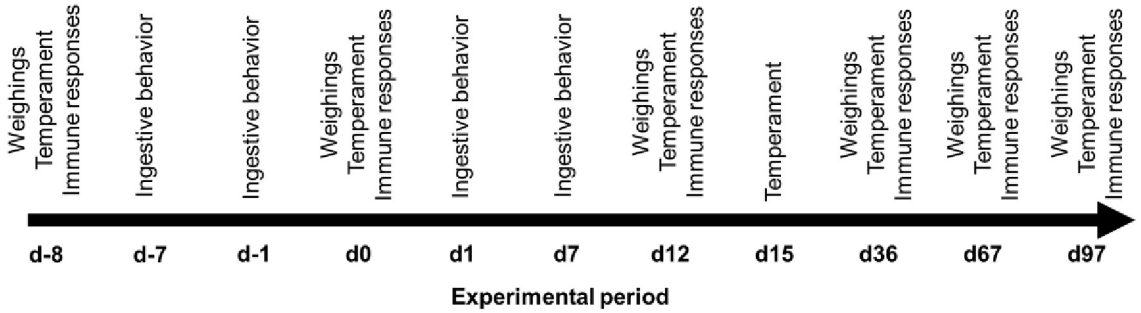

FIGURE 1 | Diagram representing the days that made up the experimental period in relation to weaning (d0).

daily gain (ADG) were determined, with ADG calculated as the weight difference divided by the days between weighings. To avoid stress and potential influences on the evaluated parameters, the calves were not subjected to food or water restriction before the weighings.

2.4 | Temperament and Ingestive Behavior

At the beginning of the experimental period (d-8) and on d0, d12, d15, d36, d67, and d97 post-weaning, the calves were led to the handling pen and confined in a chute for temperament evaluation, which was conducted by three previously trained evaluators (blinded for treatments). The evaluators assigned temperament scores to the animals based on the behavior observed during entry into and exit from the chute. For the entrance score, evaluators assigned ratings based on a scale from 1 to 5, where: 1 = calm animal with no movement; 2 = restless movements; 3 = frequent movement; 4 = constant movement, vocalization, shaking of the chute; and 5 = violent and continuous struggling [14]. The behavior at the exit of the chute was also evaluated on a scale from 1 to 3, where: 1 = animals that walked out of the chute, 2 = those that trotted from the chute, and 3 = those that ran or galloped out of the chute, according to Baszczak et al. [15].

Daily pasture behavior was assessed by visual observation on d-7, d-1, d1, and d7, from 07:00 to 17:00 h, totaling 10 h of observation per day, according to the adapted methodology of Enríquez et al. [3]. These behavioral observations were carried out by three previously trained evaluators, who recorded the following parameters: grazing—time spent by the animals selecting and apprehending forage; drinking water—time spent by the animals at the water troughs; ruminating—time spent by the animals making jaw movements without feeding; lying down—time spent by the animals in lateral or ventral recumbency; playing—time spent by the animals jumping and running in the pasture without signs of stress; nursing—time spent by the calves making suckling movements with their mouths on the udder; searching for the dam—time spent by the animals moving around the pastures; vocalizing—time spent by the calves making characteristic sounds; eating supplement—time spent by the calves at the supplement troughs; and idle—time when the animals were not performing any other activity.

The evaluators responsible for the temperament and behavioral assessments had solid experience with experimental protocols of this nature and had previously participated in studies published by our research group [6, 12, 13].

2.5 | Response to Vaccination

Blood samples were collected on d 8, 0, 12, 36, 67, and 97 via jugular venipuncture using 10-mL Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) without any anticoagulant for serum collection. Samples were stored in a thermal box with ice until processing. Upon arrival at the laboratory, tubes were centrifuged at 1200 × g for 30 min to separate the serum, which was then aliquoted and stored at −20°C until analysis. Serum rabies virus-neutralizing antibody titers were determined using the rapid fluorescent focus inhibition test (RFFIT), following the protocol adapted from Zalan et al. [16]. In brief, serial twofold dilutions of each serum sample were incubated with a standardized challenge dose of rabies virus (CVS-11 strain) and then inoculated onto BHK-21 cell monolayers. After incubation, cells were fixed and stained with fluorescein isothiocyanate-labeled anti-rabies monoclonal antibodies. The highest serum dilution that completely inhibited rabies virus infection in 100% of the cells was recorded. Results are expressed as $\log_2$-transformed titers. Positive and negative control sera were included in each assay, and all samples were run in duplicate.

2.6 | Statistical Analysis

All analyses were performed using the calves as the experimental unit. The Satterthwaite approximation was used to determine the degrees of freedom for the denominator of the fixed-effects test. The ADG was analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC, USA; version 9.4), with treatment as a fixed effect and calf (treatment) as a random effect, and BW observed on d-8 as a covariate. The other variables (response to vaccination, ingestive behavior and temperament) were analyzed as repeated measures over time using the MIXED procedure of SAS. The fixed effects included treatment, day, and the interaction between treatment × day, and the random effects included calf (treatment). The initial values were included as covariate for BW, chute scores, exit scores, and serum rabies titer. In all analyses, the covariance structure used was compound symmetry, which had the lowest values in the Akaike information criterion. Means were separated using the PDIFF function (t test), and all results were reported as least squares means (LSMEANS) followed by the standard error of the mean (SEM). Significance was considered when $p \leq 0.05$ and tendency when $p > 0.05$ and ≤ 0.10.

3 | Results

3.1 | Growth Performance

Table 1 shows the data related to the ADG of the calves based on treatments at the different weighing days. Differences between the evaluated treatments were observed in the periods from d-8 to d97 and from d0 to d97 ($p \leq 0.05$). In both periods, the calves from the CON group showed lower ADG, whereas the other treatments did not differ from each other. There was an interaction between the weighing day and treatment on the BW of the calves (Figure 2; $p = 0.10$). On days 36, 67, and 97 post-weaning, calves in the CON group had lower BW compared to the other treatments, which did not differ from each other.

3.2 | Temperament and Ingestive Behavior

The temperament of the calves at the chute entry showed a trend of difference ($p = 0.09$) between the treatments (Table 2). BAS-8 and BAS0 resulted in lower values for this parameter compared to the other treatments, which were similar to each other. The

TABLE 1 | Average daily gain (ADG, kg/day) of Nelore calves as a function of the treatments studied in the different evaluation periods.

	Treatments[1]					
Period	**CON**	**RN**	**BAS-8**	**BAS0**	**SEM[2]**	***p*-value**
d-8 to 0	−0.49	−0.77	0.09	−0.5	0.69	0.59
d0 to 15	0.53	0.74	0.78	0.70	0.15	0.61
d15 to 36	−0.11	0.46	0.14	0.56	0.26	0.25
d36 to 67	0.23	0.22	0.42	0.33	0.07	0.16
d67 to 97	0.25	0.10	0.10	−0.03	0.14	0.52
d-8 to 97	0.13[b]	0.24[a]	0.30[a]	0.23[a]	0.04	0.05
d0 to 97	0.17[b]	0.32[a]	0.32[a]	0.29[a]	0.04	0.03

Note: Means followed by different letters in the row differ from each other ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
[1]CON = topical administration of 5 mL of saline solution (0.9% NaCl) on d0; RN = restricted nursing, calves allowed to nurse for only one hour (16:30 to 17:30 h) per day from d-8 to d0; BAS-8 = topical administration of 5 mL of BAS on d-8; BAS0 = topical administration of 5 mL of BAS on d0.
[2]Standard error of the mean.

data related to ingestive behavior are presented in Table 3. Except for the time spent by the calves nursing and vocalizing, all other evaluated parameters—grazing, drinking water, ruminating, lying down, playing, searching for the dam, eating supplement, and idle—were influenced by the treatments studied ($p \leq 0.05$).

BAS-8 and BAS0 resulted in less time spent grazing compared to the CON and RN treatments, which did not differ from each other. Animals from the BAS0 and CON groups spent more time drinking water compared to the other groups. Calves subjected to RN spent less water drinking time, whereas calves treated with BAS-8 showed intermediate values, being higher than RN and lower than BAS0 and CON. Compared to the calves from the RN treatment, BAS-8 and BAS0 resulted in a greater percentage of time spent on rumination. BAS0 increased the time spent on this behavior compared to the CON treatment, which, in turn, did not differ from BAS-8.

The animals in the CON group spent less time lying down compared to those in the other treatments. Regarding the time spent playing, higher percentages of time were observed in BAS-8, whereas the other treatments did not differ from each other. Calves subjected to RN spent a higher percentage of time searching for their dam compared to the other treatments. The percentage of time spent on eating the supplement was lower in the CON treatment compared to the other treatments, and calves in the BAS0 group spent more time in idle behavior than those in the other treatments.

Figure 3 shows the intensity of nursing during the period the calves in the RN group were reunited with their dams, from d-8 to d-1. It can be observed that four minutes after being reunited with their dams, 79% of the calves were nursing, and after eleven minutes, only 43% of the calves were still nursing.

3.3 | Response to Vaccination

There was also an interaction between the evaluation day and treatment on the response to vaccination (Figure 4; $p = 0.05$). Calves in the BAS0 group had greater serum titer concentrations against rabies on d20 compared to the other treatments, which did not differ from each other.

4 | Discussion

4.1 | Growth Performance

In this study, we hypothesized that the use of RN and the BAS administration given eight days before (d-8) and on the day of weaning (d0), could improve the welfare of beef calves. This approach represents a promising strategy to replace conventional weaning, which is a significant source of stress and negatively affects the animals' health and growth performance [17–19].

This hypothesis was confirmed, as the BAS administration showed a beneficial residual effect on the animals' performance, since up to 97 days post-weaning, BAS-8 and BAS0 increased the ADG of the animals compared to the CON treatment. Similar results were reported in recent studies [1, 10, 12] with the use of BAS during the weaning of beef calves. In the studies by Cooke et al. [1] and Vieira et al. [12], the positive effects of BAS administration on ADG were more evident in the first 15 days post-administration. However, in the present study, there was no difference in the ADG of calves with the administration of BAS-8 and BAS0 during the initial 15 days. The results obtained show that BAS administration has a long-term effect, and the mechanisms involved require further investigation. The evaluation of biomarkers as indicators of stress could provide explanations for these findings.

At 36, 67, and 97 days post-weaning, the calves in the CON group had lower BW, demonstrating that weaning stress can negatively impact performance in the subsequent phases. According to Lynch et al. [18], the stress responses induced by weaning can disrupt homeostasis and compromise welfare. Furthermore, these responses can be indicators of future productivity. Capelloza et al. [11] also observed differences in BW of cattle treated with BAS only at longer days post-administration (45 days). According to Capelloza and Cooke [20], the better performance caused by BAS may be related to a lower neuroendocrine response due to reduced stress.

It is important to highlight in the present study that calves subjected to RN exhibited similar performance to those treated with BAS, and in the studies mentioned above, RN was not used. In an experiment conducted by Lay et al. [5], RN of Brahman calves at earlier life (21 days of age for about 3 weeks) reduced the stress around weaning (i.e., lower distance traveled and lower number of vocalization) with no effects on BW. Our experiment differs from the one from Lay et al. [5] because we used RN in the week immediately before weaning and not early in life as they did. These differences in RN during different ages of life in these experiments, could explain the differences in BW effects. In our experiment, the improved BW in RN—in relation to the CON treatment—calves could be associated with less stressful weaning as they were gradually adapted for this practice.

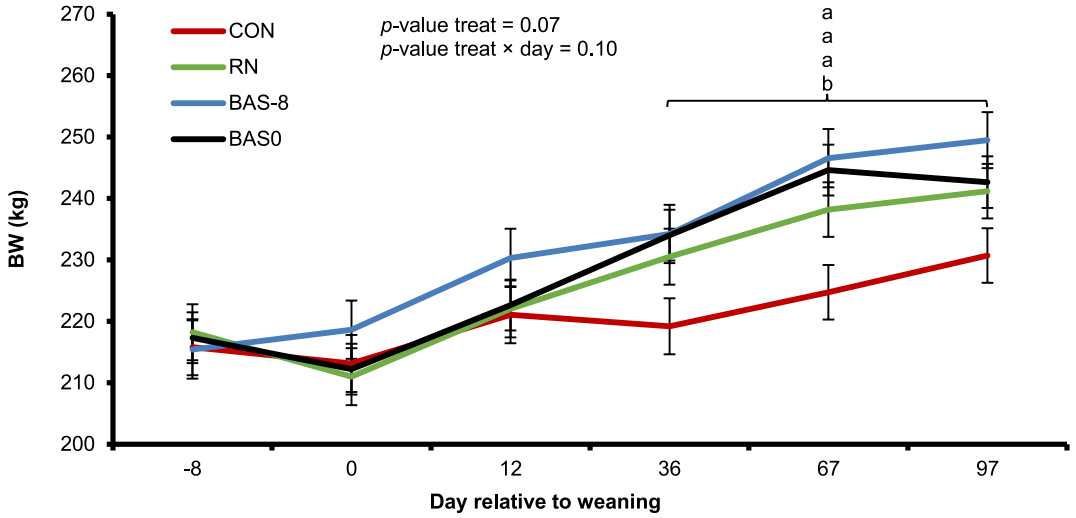

FIGURE 2 | Body weight (BW) of Nelore calves as a function of the treatments studied in the different periods of the study. CON = topical administration of 5 mL of saline solution (0.9% NaCl) on d0; RN = restricted nursing, calves allowed to nurse for only one hour (16:30 to 17:30 h) per day from d-8 to d0; BAS-8 = topical administration of 5 mL of BAS on d-8; BAS0 = topical administration of 5 mL of BAS on d-0; different letters indicate significant differences ($p \leq 0.05$) or tendencies toward differences ($p \leq 0.10$), with the order of the letters corresponding to the order of the lines in the graph. Vertical bars represent the standard error of the mean.

TABLE 2 | Temperament in the chute of Nelore calves as a function of the treatments studied.

	Treatments[1]					
Items	**CON**	**RN**	**BAS-8**	**BAS0**	**SEM[2]**	***p*-value**
Chute score, 1–5	1.25[a]	1.24[a]	1.19[b]	1.14[b]	0.05	0.09
Exit score, 1–3	1.35	1.33	1.45	1.34	0.11	0.31

Note: Means followed by different letters in the row differ from each other ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
[1]CON = topical administration of 5 mL of saline solution (0.9% NaCl) on d0; RN = restricted nursing, calves allowed to nurse for only one hour (16:30 to 17:30 h) per day from d-8 to d0; BAS-8 = topical administration of 5 mL of BAS on d-8; BAS0 = topical administration of 5 mL of BAS on d-0.
[2]Standard error of the mean.

TABLE 3 | Ingestive behavior (% of total time) of Nelore calves as a function of the treatments studied.

	Treatments[1]					
Items	**CON**	**RN**	**BAS-8**	**BAS0**	**SEM[2]**	***p*-value**
Grazing	55.3[a]	55.0[a]	51.5[b]	51.3[b]	0.89	0.001
Drinking water	1.84[a]	0.50[c]	1.23[b]	1.80[a]	0.13	< 0.001
Ruminating	2.50[b]	0.58[c]	2.74[a,b]	3.00[a]	0.20	< 0.001
Lying down	27.0[c]	29.5[a,b]	30.8[a]	28.1[b,c]	0.85	0.009
Playing	0.13[b]	0.20[b]	0.42[a]	0.12[b]	0.07	0.001
Nursing	0.64	—	0.57	0.60	0.10	0.185
Searching for the dam	0.32[b]	0.86[a]	0.07[c]	0.07[c]	0.14	0.001
Vocalizing	0.19	0.04	0.01	0.06	0.06	0.150
Eating supplement	0.41[b]	0.87[a]	0.90[a]	0.87[a]	0.13	0.020
Idle	12.1[b]	12.6[b]	12.2[b]	14.5[a]	1.04	0.010

Note: Means followed by different letters in the row differ from each other ($p \leq 0.05$) or tend to differ ($p \leq 0.10$).
[1]CON = topical administration of 5 mL of saline solution (0.9% NaCl) on d0; RN = restricted nursing, calves allowed to nurse for only one hour (16:30 to 17:30 h) per day from d-8 to d0; BAS-8 = topical administration of 5 mL of BAS on d-8; BAS0 = topical administration of 5 mL of BAS on d0.
[2]Standard error of the mean.

4.2 | Ingestive Behavior

The RN calves spent more time lying down after weaning them than CON calves, which could be related to less stress or better adaptation to the new environment. Although the calves subjected to RN spent more time searching for their mothers after weaning— which could, at first glance, be interpreted as an indication of increased stress—we believe this behavior is more

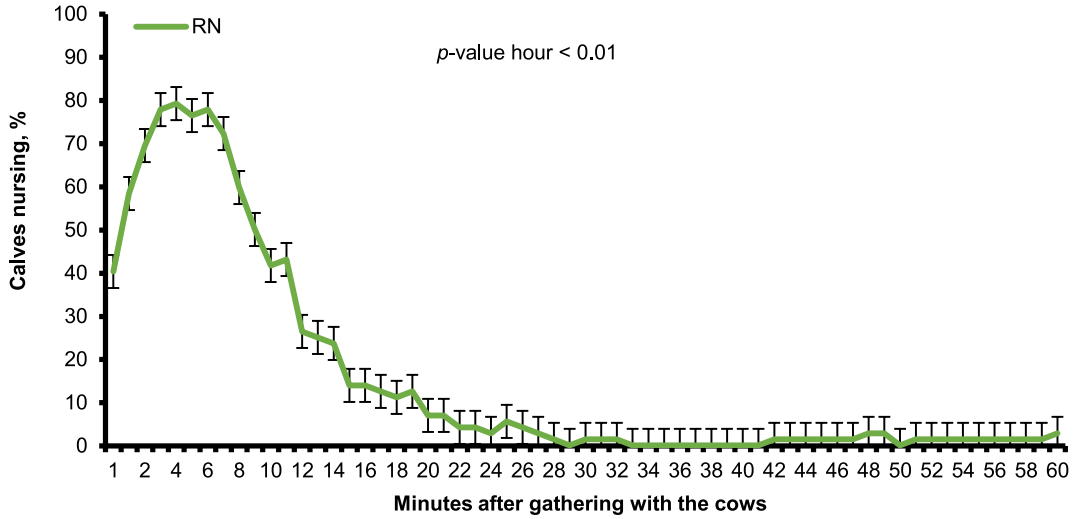

FIGURE 3 | Percentual of Nelore calves nursing after gathering them with the cows [only calves from restricted nursing (RN) treatment were evaluated]. Vertical bars represent the standard error of the mean.

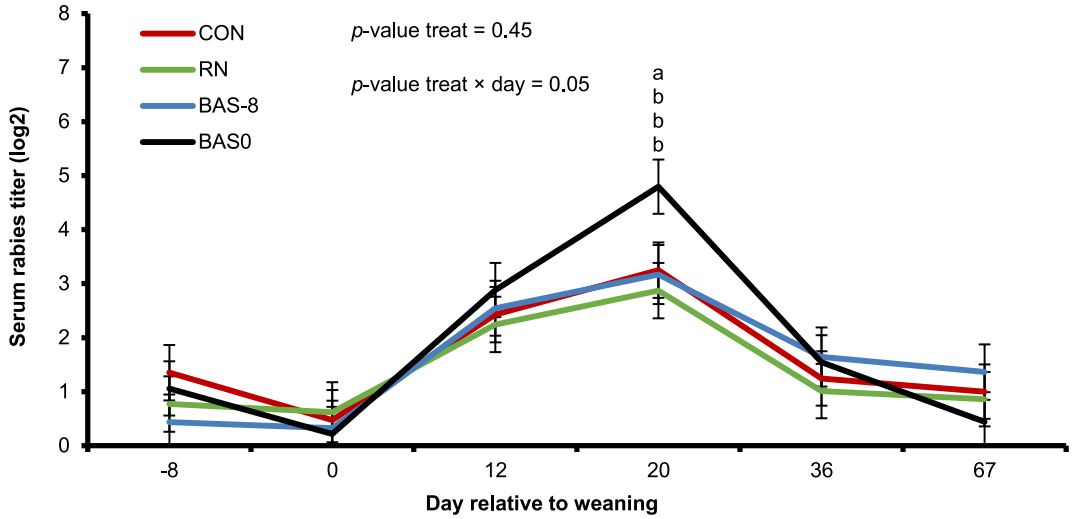

FIGURE 4 | Serum rabies titer concentration of Nelore calves that received a rabies vaccine at weaning (d0). CON = topical administration of 5 mL of saline solution (0.9% NaCl) on d0; RN = restricted nursing, calves allowed to nurse for only one hour (16:30 to 17:30 h) per day from d-8 to d0; BAS-8 = topical administration of 5 mL of BAS on d-8; BAS0 = topical administration of 5 mL of BAS on d-0; different letters indicate significant differences ($p \leq 0.05$) or tendencies toward differences ($p \leq 0.10$), with the order of the letters corresponding to the order of the lines in the graph. Vertical bars represent the standard error of the mean.

related to the previously established routine than, in fact, to an actual increase in stress levels. The regular, albeit brief, interactions with their mothers before weaning likely created behavioral expectations associated with those moments. Thus, the searching observed after weaning may reflect an attempt to maintaining this routine, and not necessarily a sign of distress. In addition, the variables of temperament and vocalization were unaffected by RN, indicating that this practice did not increase stress after weaning. In our experiment, RN calves ruminated less time than CON calves. This effect was unexpected because rumination is normally associated with grazing time [4], and grazing time was not affected in RN calves. In addition, RN calves spent less time drinking water than CON calves, and this effect was also not expected. Therefore, the effects of RN on intake, rumination, and water drinking time show preliminary results that require future investigations for confirmation or refutation, considering the inherent limitations of the present study.

It has been well documented in the literature that the BAS administration reduces stress and the emergence of antagonistic behaviors in cattle, increasing feeding time and animal performance [21, 22]. In the present study, animals treated with BAS-8 and BAS0 spent more time eating supplements, suggesting that stress in these animals was minimized, explaining the higher ADG. This justification is supported by the animals' behavior upon entering the chute, where animals from BAS-8 and BAS0 were slightly calmer compared to the other groups. Additionally, calves treated with BAS-8 spent more time playing, indicating better welfare conditions. The mechanisms by which BAS reduced the stress by weaning in our experiment were not studied. In a work conducted by Vieira et al. [13], BAS

administration at weaning reduced the subsequent serum concentration of cortisol and plasma ceruloplasmin, suggesting that BAS effectively attenuated this cascade of events.

4.3 | Response to Vaccination

Thus, minimizing stress during weaning is crucial, as the threat to homeostasis at this stage can compromise the effectiveness of a specific vaccine, interfering with the inflammatory response and the body's ability to develop an effective immune response [20]. In the present study, significant responses to the rabies vaccination were observed at 20 days, where BAS0 resulted in better responses. A greater concentration of rabies titers translates to a better protective status against possible rabies infection. In recently published studies, calves that received BAS at weaning showed higher antibody concentrations [12, 22], demonstrating the positive effects of BAS on animal health. The better immune response in BAS0 calves was probably related to less stress and less cortisol production. Cortisol affects the immune system in several ways, including reducing immune cell proliferation and differentiation, affecting cell function, and increasing cytokine expression [17].

From a practical standpoint, this suggests that the application of BAS may potentially enhance the effectiveness of vaccination, thereby increasing the animals' protection against the disease.

5 | Conclusion

The administration of BAS eight days before and on the day of weaning proved to be effective in improving performance and reducing stress in beef calves. Additionally, the use of BAS on the day of weaning improved the calves' response to the rabies vaccination. However, RN proved effective in improving the performance of calves compared to conventional weaning. Nonetheless, in terms of animal temperament, RN is less efficient than BAS. In addition, future studies are encouraged to investigate the long-term mechanisms of action of BAS on stress biomarkers during the post-weaning period.

Author Contributions

Denise E. Mussalafo: investigation, data curation, software, writing – original draft. **Marcelo Vedovatto:** conceptualization, methodology, visualization, validation, project administration, supervision. **Aylpy R.D. Santos:** writing – review and editing, formal analysis, resources, writing – original draft. **Dalton M. Oliveira:** validation, visualization, supervision, project administration. **Fabiana A.M. Sterza:** methodology, visualization. **Mariana Santos:** methodology, investigation. **Helena B.C.R. Batista:** methodology, investigation, formal analysis. **Jaíne G. Garcia:** methodology, formal analysis, investigation. **Andréa R.D.L. Souza:** validation, visualization, writing – review and editing, resources.

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Ethics Statement

All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data supporting the findings of this study are available upon reasonable request to the corresponding author.

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