Behavioural and physiological responses of male and female beef cattle to weaning at 30, 75 or 180 days of age

Odilene de Souza Teixeira a, *, Marcela Kuczynski da Rocha a, Antónia Mendes Paizano Alfora b, Vanessa Silva Fernandes a, Josiane de Oliveira Feljó b, Marcio Nunes Corrêa b, Maria Eugênia Andrighetto Canozzi c, Concepta McManus d, Júlio Otávio Jardim Barcellos a

a Beef Cattle Center for Research on Systems of Beef Cattle Production and Supply Chain, Department of Animal Production, Federal University of Rio Grande do Sul, 91540-000, Porto Alegre, RS, Brazil

b Department of Veterinary Clinics, Federal University of Pelotas, 96160-000, Pelotas, RS, Brazil

c Instituto Nacional de Investigación Agropecuaria (INIA), Programa Producción de Carne y Lana, Estación Experimental INIA La Estanzuela, Ruta 50 km 11, 39173, Colonia, Uruguay

d Institute of Biological Sciences, University of Brasilia, 70910-900, Brasília, DF, Brazil

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ABSTRACT

Weaning calves at a young age can generate great stress, but it is widely practiced in the industry despite that. So, to what extend weaning in early ages is more stressful than at later ones, in terms of the amount of stress, is currently unknown. Thus, we studied the behavioural and physiological impacts in beef calves at three ages at weaning (30, 75, and 180 days) and the consequences of the calf’s sex. Thirty-six calves were weaned and distributed in hyper-early (W30), early (W75), and conventional (W180) weaning groups, which are weaning ages that occur in practice. Behavioural activities and physiological parameters were measured on days 0, 1, 2, and 7 after weaning. Canonical component analysis (CCA) and analysis of variance (ANOVA) were used to evaluate behavioural and physiological parameters. The variables analysed were influenced by the treatment, day of evaluation and interaction between treatment × day, and without effect for sex. The younger the calf, the greater the intensity of responses to the weaning stress. In this sense, the behavioural results (percentages of observations ± SEM) demonstrate that on D0, W30 calves emitted more extreme vocalization (61 %) compared to W75 (15 %) and W180 (0%) (P < 0.001); on D1, W30 and W75 calves expressed more extreme sounds (61 % and 50 %, respectively) than W180 (21 %) (P = 0.028). Similarly, W30 and W75 calves showed a higher number of cross-sucking (P = 0.006). On D0, differences in the frequency of walking (P < 0.001) were observed, once calves of W30 (45.5 ± 6.21 %) and W75 (39.9 ± 6.03 %) spent more time in this activity than W180 ones (17.3 ± 3.80 %). Also, more visiting the feeder without eating feed events were verified (P = 0.014) on D0 (11.5 ± 2.59 %) and D1 (6.2 ± 1.65 %) for W30 when compared to W180 calves (2.6 ± 1.73 % and 0.0 ± 0.00 %, respectively). In physiological terms, a higher respiratory rate (P = 0.043) on D0 was identified for W30 calves (66.3 ± 2.90 breaths/min) than for W75 (57.8 ± 2.30 breaths/min) or W180 (47.3 ± 2.02 breaths/min). Cortisol concentrations were higher (P = 0.019) on D0 for W30 animals (15.6 ± 3.40 ng/mL) than for W180 ones (9.7 ± 1.90 ng/mL). The peak of cortisol occurred on D1 for W30 and D2 for W75 and W180. Therefore, behavioural and physiological changes at young ages indicate greater calf suffering, which may have negative consequences on a productive life.

1. Introduction

Weaning at younger ages in beef cattle is justified by the benefits in the reproductive performance of cows (Orihuela and Galina, 2019), and it is promising to higher growth rates of calves, as maternal milk only meets the nutritional requirements for performance up to 84 days of age.

* Corresponding author.
E-mail address: odilene.zootecnista@gmail.com (O. de Souza Teixeira).

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2. Material and methods

2.1. Ethical note

The management procedures performed on the calves were in accordance with the license (33439) granted by the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Rio Grande do Sul (UFRGS), Brazil.

2.2. Animals and treatments

The research was carried out at the UFRGS experimental farm, located in the city of Eldorado do Sul, Rio Grande do Sul, Brazil (latitude 30°06’ 20.7’’S and longitude 51°41’ 21.9’’W), between October 2018 and June 2019. Thirty-six Brangus calves, born in the spring of 2018, were used and divided into three treatments: i) calves subjected to hyper-early weaning (W30; n = 12) aged 31 ± 0.58 (mean ± SE) days and weight 52.8 ± 0.99 (mean ± SE) kg; ii) calves submitted to early weaning (W75; n = 12) aged 76 ± 0.68 (mean ± SE) days and weight of 79.4 ± 1.53 (mean ± SE) kg, and; iii) calves submitted to conventional weaning (W180; n = 12) aged 183 ± 0.60 (mean ± SE) days and weight of 158.5 ± 2.70 (mean ± SE) kg. Each treatment contained six male non-castrated calves and six females, born to 4.7 ± 0.41 (mean ± SE) year old cows.

2.3. Weaning, facilities and food management

On the day scheduled for weaning, after the separation of the cows and calves, the dams were taken to a paddock 2.6 km away. The same weaning management was performed for all treatments, with W30 and W75 being done in the summer (December and February, respectively) and W180 in the fall (May). The weaning groups were formed by calves from the same birth period and born from cows of the same mating season, according to the protocol described by Blanco et al. (2008).

After weaning, the calves of the same treatment were collectively allocated to a common pen (Fig. 1). This was done because group accommodation after weaning allows greater play activity and increased body weight (Valníčková et al., 2015), with gains in animal welfare (Bucková et al., 2019), in addition to replicating a common situation on farms. The surface of the pen was dirt-floor, and it was equipped with two feeders, an automatic waterer, and a shade screen made of polypropylene fabric for artificial shade (80 % shading).

The calves’ diet consisted of commercial pelleted feed (200 g/kg of crude protein (PB), 60 g / kg of crude fiber (FB) and 740 g/kg of total digestible nutrients (NDT)) and 3 cm long chopped alfalfa hay (167 g/kg PB and 456 g/kg NDF). The feed used was composed mainly of powdered whey, laminated and extruded corn and soybeans, as well as liquid molasses as a flavouring agent. Calves had ad libitum access to concentrate feed and hay, with mean total consumption of 1.05 % BW, based on dry matter, for W30 and W75 in the first 10 days, and 1.44 % BW for W180 animals. The feed was replenished twice a day, at 8 a.m. and 2 p.m, avoiding any type of interaction between the handler and the animals to do not affect the behaviour analysis. The calves had no prior experience with supplementation, and all animals (cow-calf) were in the same dietary condition before weaning, on native pasture with 2200 kg DM/ha and cultivated millet pasture (Pennisetum americanum) with 1830 kg DM/ha.

2.4. Environmental traits

Two thermometers installed at 1 m above the pen floor were used to measure the ambient temperature and relative humidity at 8 a.m., 1 p.m and 6 p.m. The average data of these variables, referring to the three moments of readings, were used to calculate the temperature and humidity index (THI), the equation proposed by Tucker et al. (2008).

2.5. Behavioural traits

Behavioural assessments were carried out for four days, on days 0 (immediately after weaning), 1, 2, and 7 post-weaning in the three treatments, from 13:30 h to 18:00 h, with an interval of 10 min between observations. These activities were not measured during the morning because they would have coincided with blood collection. The calves were identified by numbers written in marking ink on both sides of their bodies to enable the images to be read later. The evaluations were recorded by four video cameras (resolution 1080 pixels; Hikivision, Hangzhou, China) (Fig. 1). The images were then analysed by a trained evaluator who identified and recorded the behaviours of interest: Visiting the feeder without eating – approaching the feeder without eating; Eating – Feed in the mouth followed by mastication (Loberg et al., 2008); Drinking – Muzzle in contact with the water (Mitlöhner et al., 2001); Ruminating – Mastication after regurgitation (Loberg et al.,...
Walking – The four hooves of the animal were moving (Loberg et al., 2008); Standing – Animal idling, without walking (Mitlöchner et al., 2001); Lying down – Body in contact with the ground (Mitlöchner et al., 2001); Cross-sucking – One calf suckling another (regions such as ears, foreskin, navel or testicles) (de Passillé, 2001); Vocalization – Emitting sound from the mouth (Loberg et al., 2008), it was analysed by a microphone in conjunction with video.

Compiled data were considered based on data from individual animals. The experimental unit was the animal, according to a methodology also used by Smith et al. (2003) and recently by Freitas-de-Melo and Ungerfeld (2020). The cross-sucking events were observed during the same period by continuous sampling, as this behaviour does not occur often, and observing it every 10 min would not represent the pattern of this activity. Vocalization was recorded by video cameras and a microphone. The quantification of vocalizations in the groups was performed for 15 s every 10 min, being classified as absent (no vocalization), moderate (five or fewer calves emitting sound; 20 % of the sample), or extreme (six or more animals). On weaning day (D0), vocalizations were evaluated for two hours, equivalent to 13 observation moments, and on days 1, 2, and 7 for four and a half hours, totaling 28 moments.

2.6. Physiological traits

In the morning, on days 0, 1, 2, and 7 post-weaning, the calves were taken to the handling facility where the animals’ respiratory and heart rates were measured for one minute using a stethoscope (Littmann classic III, USA), as well as the rectal body temperature, using a digital clinical thermometer (Incoterm, Brazil). These procedures were performed after blood collection by trained personnel who manually handled the animals calmly and silently.

2.7. Collection and analysis of blood parameters

Blood collection started at 08:00 h after the calves had been fed. On days 0, 1, 2, and 7 post-weaning, the animals were immobilized in the corral to collect blood through puncture of the jugular vein. Cortisol analyses were performed using 10 mL vacutainer tubes (BD, Franklin Lakes, NJ, USA) with a clot activator. The samples were centrifuged (2000 × g for 20 min) and frozen in a cylinder containing liquid nitrogen (-196 °C). For the analysis of total plasma proteins and fibrinogen, blood was collected in 4 mL vacutainer tubes tubes (BD, Franklin Lakes, NJ, USA) containing ethylenediaminetetraacetic acid (EDTA, K2, 7.2 mg per tube). After collection, the samples were frozen in liquid nitrogen and sent to the laboratory. It was used for total plasma proteins an automatic counter (Procyte DX; Idexx, Westbrook, Maine, USA), for fibrinogen concentration in blood plasma the heat precipitation method (Willard and Tvedten, 2004), and for cortisol the chemiluminescence method using a commercial kit (Beckman Coulter), which was read by the Access II equipment (Beckman Coulter, Brea, California, USA).

2.8. Experimental design and statistical analysis

The experimental design was completely randomized, with repeated measures in time (days). The calves were considered as experimental units (n = 12, replicates). From the set of behavioral and physiological variables of the calves, patterns and similarity of responses were investigated for the three ages at weaning, using a canonical component analysis (CCA). This analysis was used as an initial global diagnosis, aiming to understand, at first, the set of variables in a grouped way (without distinguishing the variables individually). Also, the THI and the age of the cow in years were entered as covariates on this analysis. Thereby, the CCA established the ordering of the data, the formation of the groups of weaning and similarities between them, as well as the evaluated days, controlling factors associated with climate and possible differences related to the dam. The validation of these groups was carried out by analyzing permutational multivariate variance (PERMANOVA) with 9999 repetitions. The CCA and PERMANOVA analyses were operationalised using the PAST 3.0 software (Hammer et al., 2001), using 5% as the level of significance.

After CCA had been performed, the variables were verified separately using the general linear model in an analysis of variance with repeated measures (ANOVA) to evaluate the evolution of each variable over the different days. Simple linear regressions were performed within each treatment to assess the effect of THI on behavioural and
physiological variables. Pearson’s correlation was used to determine the association between the evaluated behaviours. The model included the effect of age at weaning (W30, W75, and W180), and days (0, 1, 2, and 7 post-weaning) were considered fixed factors and the interaction between them and animals was considered a random factor. Dunnet’s T3 test was used to compare the means. The vocalization data were analysed using the Chi-Square test and the cross-sucking events using the Generalised Linear Model (GLM), considering the Poisson linear distribution. For these analyses, a 95% confidence interval was used. These analyses were carried out using SPSS 20.0 software (IBM, 2011, Armonk, New York).

3. Results

3.1. Effect of age at weaning on behaviour and physiological parameters

Response patterns were observed for the three ages at weaning based on the degree of similarity between the groups (Fig. 2). Thus, W30 calves were more similar to W75 ones in the post-weaning period, which can be geometrically evidenced by the overlapping circles of these two groups (P = 0.597). In contrast, W30 and W75 calves were different from W180 (P < 0.0001), marked by the distance between the circles. In addition to the treatment effect (P = 0.007), day was also significant (P = 0.022). The calf’s sex was not associated with changes in the analysed variables (P = 0.574). The age of the calf’s mother did not influence the behavioural and physiological responses (P = 0.776). On the other hand, the THI had a significant effect (P = 0.001).

3.2. Implications of age at weaning on the behaviour and physiological parameters of the calves over time

As the calves grew older, the behavioural and physiological indicators became more constant, regardless of weaning age. Greater dispersion and significance of the circles can be seen in the first days after weaning compared to D7 (Fig. 3). The W30 calves exhibited differences on days 0, 1 and 2 (P < 0.001), with no change in the response pattern comparing days 2 and 7 (P = 0.376). The W75 calves showed changes in the characteristics every day (P < 0.05). For W180, similarities were observed on days 0 and 2 (P = 0.056) and changes on the other days (P = 0.004). In addition, the THI influenced treatments W75 (P = 0.001) and W180 (P = 0.005). These results allow us to infer that the first days of weaning are the most challenging, but behavioural activities and physiological parameters decrease their dispersion as the days pass.

3.3. Behavioural changes at different weaning ages

There was an interaction between the treatment and the post-weaning day for activities of visiting the feeder without eating (P = 0.014), feeding (P = 0.016), walking (P < 0.001), lying down (P < 0.001) and ruminating (P < 0.001) (Fig. 4).

3.3.1. Visiting the feeder without eating

The frequency of visiting the feeder without consuming food was higher for W30 calves on D0 (11.5 ± 2.59 %) and D1 (6.2 ± 1.65 %) post-weaning when compared to W180 calves (2.6 ± 1.73 % and 0.0 ± 0.00 %, respectively) (P = 0.001). The W75 calves showed an intermediate behaviour to the other groups on D0 (7.7 ± 1.80 %) but were similar to W180 on D1. As the days post-weaning advanced, visits to the feeder decreased, since on days 2 and 7 this activity practically ceased in all treatments (Fig. 4).

3.3.2. Eating

The frequency of animals observed eating was about four times lower for calves W30 (7.7 ± 3.41 %) and W75 (9.1 ± 4.12 %) on D0 compared to W180 (38.5 ± 8.52 %) (P = 0.016). On that day, seven W30 calves did not feed, as well as five animals from W75 and one from W180. However, W180 calves decreased the frequency of visits to the feeder with eating activity (16.4 ± 2.30 %) on D1 and were statistically equivalent to W75 calves (12.5 ± 3.48 %) while W30 animals had the lowest frequency (6.8 ± 1.78 %). Still on D1, food was negatively associated with walking (r = -0.58; P = 0.01) and visiting the feeder without eating (r = -0.50; P = 0.01).

From D1, there was an increase in feeding activity regardless of treatment. However, the W180 calves achieved a higher frequency on D7 when compared to W30 ones (Fig. 4). Also, the higher frequency of feeding by the older animals was related to a 27 % increase in consumption, calculated based on the live weight of the calves in the first ten days after weaning. Feeding activity is also explained in part by THI in all treatments (P < 0.01), with 8% (W30), 5% (W75), and 18 % (W180) of the variation being associated with this variable.

3.3.3. Draining

The age at weaning of the calves did not influence their searching for the drinking fountain and water intake (P = 5.25), but evaluation day did (P = 0.010). On D0, calves attended the water point 53 % more than on D1. In the days following weaning, the animals reestablished this activity and resembled D0 (Fig. 4).

3.3.4. Walking

The activity of moving around was the main activity performed by the calves of W30 (45.5 ± 6.21 %) and W75 (39.9 ± 6.03 %) on the day of weaning, and was greater than the W180 calves (17.3 ± 3.80 %; P < 0.001 (Fig. 4). On the day after weaning, there was no difference in the age of the calves for this activity. On D2, the W30 calves reduced the activity of walking (4.2 ± 1.23 %) compared to W75 (15.2 ± 4.53 %), and W180 calves were similar to the two groups (8.6 ± 1.20 %).

3.3.5. Lying down

The W75 calves remained at rest longer on D0 (7.0 ± 2.19 %) and D1 (29.5 ± 2.16 %) than W30 calves (D0: 1.3 ± 0.86 % and D1: 9.5 ± 3.24 %) and W180 (D0: 0.0 ± 0.00 % and D1: 14.0 ± 2.96 %) (P = 0.001; Fig. 4). On D2, calves in the W30 group had a higher frequency of lying down (the most frequent activity made on that day by the animals of this treatment), in contrast to the W180 calves, which remained for a short time at rest. On that day and D7, this variable was negatively associated with standing (r = -0.70; P = 0.01) (r = -0.62; P = 0.01). On D7, W180 calves showed the lowest frequency of time in this activity compared to W75. The W30 calves had an intermediate behaviour to the W75 and W180 calves.
3.3.6. Standing

The highest frequency in this activity was for W180 calves with a total mean of 38.4 ± 2.44 % compared to W30 (22.9 ± 0.35 %) and W75 (23.0 ± 1.88 %) (P = 0.006; Fig. 4). However, 6% of the variation in this activity is due to THI on the W180 calves (P = 0.038), i.e., as the THI increased, the animals remained standing for more time. Even though on D1 and D2 the calves remained standing longer, on D7 there was a decrease in the duration of this activity. Even with the difference between W75 and W180, the pattern was similar between these ages at weaning.

3.3.7. Ruminating

Rumination was higher (P < 0.001) for W75 calves (9.1 ± 2.28 %) on D0 when compared to W180 (0.6 ± 0.64 %), which had similar behaviour to W30 (4.5 ± 2.75 %). This response was inversed on D1, once W30 calves ruminated more (18.4 ± 2.23 %) comparing to the W75 (5.9 ± 2.03 %) and W180 (3.9 ± 1.03 %) ones. Such changes disappeared in the days following weaning, both for treatments and in the evolution of days (Fig. 4).

3.3.8. Vocalization

Age at weaning influenced the type of vocalization on D0 (P < 0.001) and D1 (P = 0.028), with no difference for the other days (Fig. 5). On D0, W30 calves emitted more extreme vocalization events (61 %) compared to W75 (15 %) and W180 (0%); on D1, calves W30 and W75 expressed more extreme sounds (61 % and 50 %, respectively) than W180 (21 %). The group W180 emitted more moderate vocalizations on D1 (54 %) than W75 (43 %) and W30 (29 %) ones. W180 calves were those that least vocalized compared to W30. From D2, no difference was observed between groups (P = 0.333), with the total absence of this behaviour observed on D7 after weaning.

3.3.9. Cross-sucking

Cross-sucking events differed between treatments. The W30 and W75 animals showed a higher number of cross-sucking (P = 0.006), with this activity reaching up to 7 events for calves W30 and 6 for W75, but the most common were 1, 2, or 3 sucking events for these treatments.

3.4. Physiological parameters

There was an interaction between the treatment and the post-weaning day for respiratory rate, heart rate, rectal body temperature, and cortisol levels (P < 0.001).

3.4.1. Respiratory rate

The respiratory rate was higher for W30 calves (P = 0.043) (66.3 ± 2.90 breaths/min) than for W75 (57.8 ± 2.30 breaths/min) or W180 (47.3 ± 2.02 breaths/min) calves on D0. On D1, W30 animals (P = 0.030) (63.0 ± 3.50 breaths/min) had a higher number of breaths per minute compared to the W180 animals (49.3 ± 1.66 breaths/min). These differences disappeared on D2. On D7, the W75 animals (71.6 ± 2.40 breaths /min) had the highest (P = 0.025) rates compared to W30 (61.7 ± 3.30 breaths /min) and W180 (54.0 ± 1.60 breaths/min) (Fig. 6).

3.4.2. Heart rate

Heart rate did not change on D0 for any age at weaning. On D1 and D2, W30 (62.7 ± 3.49 and 87.7 ± 6.35 beats/min, respectively) and W180 (74.7 ± 3.56 and 87.3 ± 3.15 beats/min, respectively) calves were similar to each other (P = 0.129 and P = 0.615, respectively), but they differed from W75 (92.3 ± 4.98 and 108.0 ± 4.64 beats/min, respectively) (Fig. 6). These differences no longer existed on D7 post-weaning. THI was responsible for 5% in W30 (P < 0.001) and 9% in
3.4.3. Rectal body temperature

On the day of weaning, W30 calves showed higher rectal body temperature (39.5 ± 0.06 °C) compared to W75 (P = 0.01) (39.2 ± 0.01 °C) and W180 (P < 0.001) (39.1 ± 0.07 °C) (Fig. 6). On D1, these changes disappeared, but returned on D2, with W180 (39.3 ± 0.13 °C) having a higher (P = 0.05) body temperature than W30 (38.9 ± 0.08 °C). On D7, calves W75 had the highest temperature in relation to W30 (P = 0.03) and W180 (P < 0.001). This measure proved to be similar to respiratory rate responses over time, especially for W75 and W180 calves. For W30 and W75, 37% and 3% of the body temperature variation, respectively, were influenced by THI (P < 0.001).

3.4.4. Total proteins, plasma fibrinogen, and cortisol

Weaning age altered total serum proteins (P < 0.043), with higher levels for W180 animals (69.4 ± 0.38 g/L) compared to W30 (65.4 ± 0.10 g/L) and W75 calves (64.1 ± 0.29 g/L). Plasma fibrinogen levels changed on D2, being higher (P = 0.013) for W30 calves (4.8 ± 0.30 g/L) when compared to W180 (3.5 ± 0.36 g/L). The W75 animals were similar to the other groups on that day.

Cortisol concentrations were higher (P = 0.019) on D0 for W30 animals (15.6 ± 3.40 ng/mL) than for W180 (9.7 ± 1.90 ng/mL) and similar to W75 (P = 0.185) (11.8 ± 2.32 ng/mL). On D1, the levels increased (P < 0.001) for W30 calves (21.7 ± 4.06 ng/mL), being higher than that presented by W75 (6.8 ± 0.59 ng/mL) and W180 (7.1 ± 1.33 ng/mL). On the other days, no differences were observed. The peak of
cortisol occurred on D1 for W30 and D2 for W75 and W180 (Fig. 7).

4. Discussion

From the comparison of different ages at weaning of calves, the influence of early (30 or 75 days) or traditional (180 days) separation on behavioural and physiological responses was evaluated. Our hypotheses were that calves weaned at young ages would present more significant stress in the immediate period after weaning and, consequently, greater impairment of activities important for survival and development. Also, based on the studies by Reinhardt and Reinhardt (1981) and Stehulová et al. (2013), we hypothesised that male calves would suffer more significant consequences of weaning than females. Contrary to what was expected, the calf’s sex did not exert an effect. Our results showed that the younger the calf is, the greater the intensity of responses to weaning stress. These reactions are mainly characterised by an increase in extreme vocalizations, more walking, less frequency in feeding, a higher percentage of visits to the feeder without eating food, a greater number of cross-sucking events, as well as increased respiratory rate, fibrinogen, and cortisol levels. Nevertheless, these changes are mostly associated with D0 and D1 and lose intensity after D2 post-weaning.

The changes in vocalizations and the greater frequency in walking of the younger calves, which was 2–3 times greater and took up almost 50% of the time, when compared to the older animals, suggest that the young animals face inadequate welfare conditions after weaning. It happens possibly because they quickly identified the mother’s absence and then vocalized with greater intensity while walked through the pen trying to locate it. These walking and vocalization behaviours are exhibited after separation or weaning in cattle (Loberg et al., 2006; Price, 2008), with an emphasis on vocalization (Stehulová et al., 2017).

Fig. 5. Effect of treatment and evaluation day on types of vocalizations by beef calves submitted to three ages at weaning. (Day 0: * P < 0.001; Day 1: * P = 0.028). W30: hyper-early weaning; W75: early weaning and W180: conventional weaning.

Fig. 6. Effect of treatment and day on the physiological parameters of calves weaned at different ages. W30: hyper-early weaning; W75: early weaning and W180: conventional weaning.
The marked behavioural activity of movement and vocalization in the first days seems to have caused the increase in the period of lying down on the following day, maybe as an indication of physical exhaustion. Price et al. (2003) associate this non-reactive behaviour of lying idle with adaptation to a new environment, new social relationships, and habituation to the absence of maternal care in weaned calves.

On the other hand, older calves took longer to respond to their mother’s absence. In practice, it is seen that, over time, the cow-calf pair becomes more distant throughout the day (Vitale et al., 1986), and natural weaning occurs around 10 months (Reinhardt and Reinhardt, 1981). At this time, there may be a less strong link between the calf-dam pair (Haley et al., 2005). Therefore, older calves take longer to respond to the lack of maternal presence, which may indicate that weaning is less stressful in these animals (Smith et al., 2003).

The vocalization emitted by animals is fundamental, as it is an indicator of welfare (Padilla de la Torre et al., 2015). It can be used to demonstrate distress in the post-weaning period, possibly motivated by the intention to find the mother and receiving maternal care (Weary et al., 2008), as well as satiating hunger (Johnsen et al., 2018). Thus, the intensity of vocalization will depend on the needs of young calves for these aspects (Stěhulová et al., 2017) since they are more pronounced at early ages, as occurred in the present research. This is because as the calf grows, the number of feedings decreases, as does the total time in this activity (Reinhardt and Reinhardt, 1981; Castanheira et al., 2013). It was also shown that the same occurs with maternal care aimed at protecting the calf (von Keyserlingk and Weary, 2007).

Lambertz et al. (2015) also found that calves weaned at eight months of age vocalized less during the first three days after weaning (average of 7 calls/10 min) than calves weaned at six months (average of 4 calls/10 min). According to these researchers, older calves already have social connections with other animals and have learned to look for feed sources other than milk. In addition, in dairy calves, it is observed that the more independent they were from maternal milk at the time of total separation, the less high-pitched vocalizations occurred (Johnsen et al., 2018). This less dependence on maternal nursing of older calves was also observed in this study, as the animals in the W180 treatment stayed longer at the feeder and had higher food intake. Knowing that the regulation of food consumption is carried out by the neuroendocrine system, which is sensitive to stress extensions, the appetite of young animals may have been reduced due to the recognition of stressors by the central nervous system (Matteri et al., 2000). As a reflex, the low rate of time in feeding is considered an expression of distress at weaning (Price et al., 2003).

On D1, there was a decrease in the feeding frequency for W30 and W180 calves, and it was also seen for the water intake frequency. According to Hogan et al. (2007), these activities are positively associated, as water intake stimuli are motivated by food consumption. These findings cause challenges to the health of young calves once feeding is essential to ensure survival post-weaning, as body reserves are low at this age (Silva and Bittar, 2019). In line with the present study, the lower the age of milk calves and piglets at weaning, the less time these animals spent consuming solid food, which resulted in reduced performance (Worobec et al., 1999; Eckert et al., 2015). In this circumstance, it is recommended that calves are monitored post-weaning to assess whether they are effectively consuming food. Reported in two species - pigs and cattle, this behaviour should happen instantly after weaning, as a delay at the beginning of ingestion results in poor performance (Worobec et al., 1999; Orihuela and Galina, 2019) and increases mortality (Rasby, 2007). The inspection has to be thorough, as we observed that the youngest calves visited the feeder more frequently without eating. Remote monitoring devices can be used to assess feeding behaviour and identify in advance calves at risk of developing health problems (Belaid et al., 2020).

Although none of the groups evaluated had previously been adapted to the diets provided in feeders, older calves were more accustomed to consuming pasture with their dams. Therefore, because they already recognise roughage, such as the presence of alfalfa hay, there may have been a stimulus for roughage intake. Nevertheless, when evaluating cognitive aspects of dairy calves separated from their mothers at 42 days, researchers demonstrated that even animals trained to receive food before weaning showed a negative response bias after separation, lasting 2.5 days (Daros et al., 2014). Thus, it is not clear whether the use of techniques such as creep-feeding, which would help the calf recognise the feeder as a feeding place, can minimise visits to the trough without ingesting food during weaning.

Age at weaning led to an increase marked in rumination activity for W30 calves. This is not fully understood since food consumption decreased and, with the change from liquid to solid food, a reduction in rumination activity was expected. A similar response was reported by Lopeiato et al. (2020), who weaned Simmental calves at 45 or 60 days of age and observed a difference in rumination time so that there was a surprising increase right after weaning for both groups and then a gradual decrease. The result found in our research may probably be related to the physiological changes of the gastrointestinal system in young calves since the highest rumination was observed in W30 calves. At thirty days of age, the stomach compartments of calves are in the process of development (Church, 1974) and, despite the existence of rumination activity from the second week of life (Swanson and Harris, 1958), the marked increase in tissues and ruminal content only happens between the fourth and sixth week (Kesler et al., 1951). Therefore, the occurrence of instability in the first and second months may result from the state of animal development itself (Lengemann and Allen, 1955).

The separation of the calves (W30 and W75) motivated the appearance of cross-sucking. Although abnormal, such activity is associated with weaning at young ages (de Passillé, 2001) since maternal deprivation in periods of greater dependence on the dam causes the...
appearance of stereotyped behaviours. This is due to the frustration of not being able to suckle or have contact with the mother (Latham and Mason, 2008). Weaning in dairy calves at six weeks of age increased 75% of non-nutritive oral behaviours (crossed suckling and/or licking) compared with calves weaned at eight weeks (Eckert et al., 2015). Thus, one of the concerns with the incidence of this activity refers to the decrease in energy consumption (de Passille et al., 2010), which can lead to weight loss, a fact that is undesirable in production systems.

As a rule, the behavioural expressions of animals are closely correlated with physiological responses (Milthöner et al., 2001). In this context, it was found that the increased walking frequency of W30 calves caused higher respiratory rate and, consequently, rectal body temperature. The temperature range observed between treatments suffered a variation from 38.9 °C to 39.9 °C, with the maximum limit observed for the W75 animals, while the respiratory rate followed this same increase. In 6-month-old calves, the rectal temperature measured in the summer in the morning varied from 39.1 °C to 39.6 °C (Theurer et al., 2014), values close to those found here.

Although the animals in the present study were housed in a pen with artificial shade, the body temperature and heart rate were influenced by weather conditions. These measures were greater for the younger animals (W30 and W75), which was expected, as the climatic conditions were more severe at these times. However, this could have been a problem since the rhythm of body temperature is only established after the second month of life (Piccione et al., 2003) once the temperature control center in the hypothalamus is immature in young animals. Therefore, physiologically, these animals were not prepared to perceive and restore homeostasis after thermal stress. As expected, total plasma protein concentrations were higher for calves weaned later, as there is an increase in protein concentration (immunoglobulins) with advancing age (Bueno et al., 2003). On the other hand, younger calves (W30 and W75) showed higher cortisol concentrations and plasma fibrinogen. This increase in cortisol is the physiological representation of the behaviour exhibited by calves. The first days post-weaning was characterised by restlessness and suffering for the young calves, whereas, in the older ones, this increase occurred later and is also accompanied by changes in behaviour.

Murata (2007) described that the physiological relationship between stress and acute-phase proteins is based on a concept of neuroendocrine-immune network. The signals from sensory organs in response to stress activate the neuroendocrine center, stimulating the release of catecholamines and glucocorticoids (cortisol), which, in turn, directly and/or indirectly activate the production and release of acute-phase proteins in the liver of stressed animals. Thus, fibrinogen and other acute-phase proteins are possible markers in assessing stress during cattle management (Arthington et al., 2003; Qiu et al., 2007). Fluctuations in concentrations demonstrate the gravity of the stress on the animal (Murata et al., 2004). Similar to that found in the present study, Hickey et al. (2003) found higher concentrations of fibrinogen for weaned calves than non-weaned calves, with an increase at 48 h post-weaning and a decrease at 168 h.

Given the above, it is clear that precautions are necessary to establish weaning at 30 days of age. At very young ages, behavioural and physiological signals occur that express moments of anguish for the animals, which can have considerable consequences during the productive life and, therefore, is an emerging topic of investigation. Changes in weaning management can be evaluated as an attempt to minimise stress. The supply of concentrated food before weaning may help reduce visits to the feeder without consuming food as a way to stimulate eating behaviour (Carvalho et al., 2019). More research is needed to mitigate the low frequency of feeding in the post-weaning period in young calves. It is also relevant to investigate new separation methods, such as procedures that allow visualisation and/or physical contact between the cow-calf pair (Price et al., 2003; Taylor et al., 2019).

Finally, it has not yet been reported how the female calves from hyper-early weaning will react with their future progenies. As they were weaned at extremely young ages, certain learning by imitation actions, such as the act of grazing and the choice of suitable forage for grazing, were not assimilated by the calves at weaning. Therefore, it is essential to observe the maternal behaviour of these animals and thereby contribute to the development of a technique with minimal adverse effects on the calves accompanied by improvements in the productive and reproductive indices of the cow-calf operation.

5. Conclusions

Age at weaning implies behavioural and physiological changes in the calves. Calves weaned before three months of age demonstrate increased vocalization, increased demand for the mother, limited feeding frequency, increased cross-sucking, and higher cortisol levels compared to 6-month-old calves, especially in the first days after weaning. These events indicate the need to adapt management, feeding, or facilities to this practice to minimise the negative impacts of weaning at young ages.

Declaration of Competing Interest

The authors report no declarations of interest.

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References


