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Behavioural responses of 6-month-old beef calves prevented from suckling: influence of dam's milk yield

M. J. Hötzel[A,D], *R. Ungerfeld*[B] *and G. Quintans*[C]

[A]Laboratório de Etologia Aplicada, Departamento de Zootecnia e Desenvolvimento Rural, Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.
[B]Departamento de Fisiología, Facultad de Veterinaria, Montevideo, Uruguay.
[C]Instituto Nacional de Investigación Agropecuaria, Treinta y Tres, Uruguay.
[D]Corresponding author. Email: mjhotzel@cca.ufsc.br

Abstract. The objective of the present experiment was to compare the behavioural response of calves reared by cows of different milk yields to the prevention of suckling with the aid of nose-flaps that allowed the maintenance of social contact with the dam. Twenty Hereford or Hereford × Angus crossbred primiparous cows from a single herd remained with their calves suckling until the beginning of the experiment, in late summer–early autumn. Milk production was determined monthly during the lactation period and the pairs were selected according to the cows' milk production, for higher or lower milk yield (HMY and LMY, respectively). On Day 0, suckling was prevented by placing a nose-flap anti-suckling device on each calf for 11 days. Calf behaviour was recorded from Day –3 to Day 5, using instantaneous sampling of individual animals in each group, and the calves were weighed monthly since birth, and on Days –8 and 11. Bodyweight profiles for HMY and LMY calves were similar until Day –8; from then on, up to Day 11, HMY calves lost weigh, whereas LMY calves gained weight. Suckling frequency before nose-flaps were fitted was similar for HMY and LMY calves. There was no relationship between the cow's milk yield and the changes observed in behaviour. Apart from playing, all behaviours were affected by prevention of suckling. The distance between the calves and the dam, and the frequency of grazing and rumination, decreased after nose-flaps were placed, whereas vocalisations, suckling attempts, walking and standing increased. In conclusion, the behavioural responses to prevention of suckling did not differ between the 6-month-old calves reared by cows with high yields and those with low milk yields. The behavioural response of calves that were prevented from suckling but were kept with the dams indicates that the cessation of suckling contributes to the weaning distress of 6-month-old beef calves.

Introduction

Natural weaning is a process whereby the young gradually achieve social and nutritional independence from the dam (Weary *et al.* 2008). In cattle, the calf and the dam maintain some social contact for some months after cessation of suckling, which in natural conditions occurs from 7 to 11 months of age (Reinhardt and Reinhardt 1981). In contrast, in farming conditions calves are often abruptly weaned – separated from the cow and prevented from suckling – at a moment when the social and nutritional transition is not completed, resulting in physiological (Lefcourt and Elsasser 1995; Hickey *et al.* 2003) and behavioural (Veissier and Le Neindre 1989; Price *et al.* 2003; Haley *et al.* 2005; Enríquez *et al.* 2010) responses that indicate distress.

To develop methods that reduce weaning distress in farm animals, it is necessary to understand how the different factors involved in weaning contribute to weaning distress (Weary *et al.* 2008). It is not known to what extent the behavioural responses observed in weaned calves reflect responses to separation from the dam or to cessation of suckling – which include both changes in hormone concentrations and milk intake (Weary *et al.* 2008). For example, social attachment to the dam is still strong at the age when most beef calves are weaned in extensive pastured systems, about the sixth month of life, and is preserved in 8-month-old calves even after 3 weeks of separation (Veissier and Le Neindre 1989; Veissier *et al.* 1990). In one study, it was observed that 6-month-old calves prevented from suckling for 17 days with the use of nose-flaps displayed an increase in vocalisations, fence-line pacing, and a loss in average daily gain (ADG) when eventually separated from the cow (Enríquez *et al.* 2010). As the loss of milk supply had occurred 17 days earlier, it was concluded that at that age the separation from the dam contributes to the emotional distress of weaning in calves. In a recent study, we found that the response to abrupt weaning at 6 months of age was greater in calves that were reared by cows of higher milk yield; calves from lower-producing cows spent more time walking and standing and, whereas both groups reduced the time spent grazing, calves reared by higher-producing cows took more days to return to baseline frequencies, and spent more time lying (Ungerfeld *et al.* 2009). Nonetheless, as the higher milk yield was related to differences in pasture availability, and the calves that were reared by cows with higher milk yield were ~30% heavier than those reared by cows with lower milk yield, the responses observed could also be explained by differences in the

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developmental stage at weaning (e.g. rumen development, or greater activity levels due to more mature physical development).

In the present study, we compared the behavioural response of calves reared by cows of different milk yields to the prevention of suckling with the aid of nose-flaps, which allows the maintenance of social contact with the dam. To avoid confounding effects arising from differences in the development of calves, we selected high and low milk-producing cows from a single group of cows reared in the same pasture. We predicted that the behavioural response to being prevented from suckling would be greater in 6-month-old calves reared by cows with higher milk yields.

Materials and methods

Animals and management

The experiment was carried out in Palo a Pique Experimental Unit of the Instituto Nacional de Investigación Agropecuaria (INIA), Treinta y Tres (34°S), Uruguay, during March 2009 (late summer–early autumn), with a herd of 53 primiparous Hereford or Hereford × Angus nursing beef cows and their calves born between 11 September and 12 October 2008. During the experiment, cows and calves grazed together on native pastures. Calves and cows were identified with numbers painted on both sides of their body with non-toxic paint.

Milk production

Milk production was assessed monthly from October 2008 to March 2009. At ~0500 hours, cows were separated from their calves and injected intramuscularly with 10 IU of oxytocin (Hipofamina, Laboratorio Dispert SA, Montevideo, Uruguay). Two minutes after the injection, cows were milked with a portable milking machine. Calves were fitted with nose-flaps and remained with their dams in the same paddock. The following day, at 0600 hours, cows were milked again using the same protocol and the milk obtained was weighed.

Twenty cow–calf dyads were selected from the herd to be used in the experiment according to the cows' milk production, which on the last milking ranged between 1.14 L and 6.24 L. The mean value for the lactation recordings from each cow between October 2008 and March 2009 was calculated, and the 10 dyads with the highest 20% (HMY group), and the 10 dyads with the lowest 20% (LMY group) milk yield were used. Main characteristics of calves from each group are presented in Table 1, and the milk yield of HMY and LMY cows is presented in Fig. 1*a*.

Animals were moved to two separate paddocks of 6 ha each, and five dyads from HMY and five from LMY group were allocated in each paddock. Seven days later (30 March), suckling was prevented by placing a nose-flap anti-suckling device (El destete, Buenos Aires, Argentina; see picture in Enríquez *et al.* 2010) on each calf (Day 0) for 11 days. Water was available ad libitum and no additional supplement was offered to the calves.

Table 1. Main characteristics of calves reared by cow's of high (HMY) and low (LMY) milk yield

Characteristic	HMY	LMY
Bodyweight at birth (kg)	38.7 ± 1.8	38.0 ± 1.3
Gender ratio (male : female)	6 : 4	4 : 6
Cow's milk production (19 March) (L/day)	5.06 ± 0.24	3.16 ± 0.34
Bodyweight (18 March) (kg)	173.6 ± 5.5	190.8 ± 6.0
Age on Day 0 (days)	178.9 ± 1.7	176.7 ± 2.1

Calf bodyweight

Calves were weighed monthly since birth, and on Days –8 and 11. ADG between the last two weighings was calculated for each group, and is expressed as kg/day.

Behavioural recordings

Behaviours were recorded using instantaneous sampling of individual calves in each group every 10 min. Data were recorded 36 times per day during three observation periods from 0800 to 0950 hours, from 1200 to 1350 hours, and from 1600 to 1750 hours. Recordings were carried out from Day –3 to Day 5, except on Day 0. Two positions and six mutually exclusive behaviours were recorded (Table 2). Vocalisations were recorded for a 30-s period every 10 min, using 0/1 sampling (Lehner 1996).

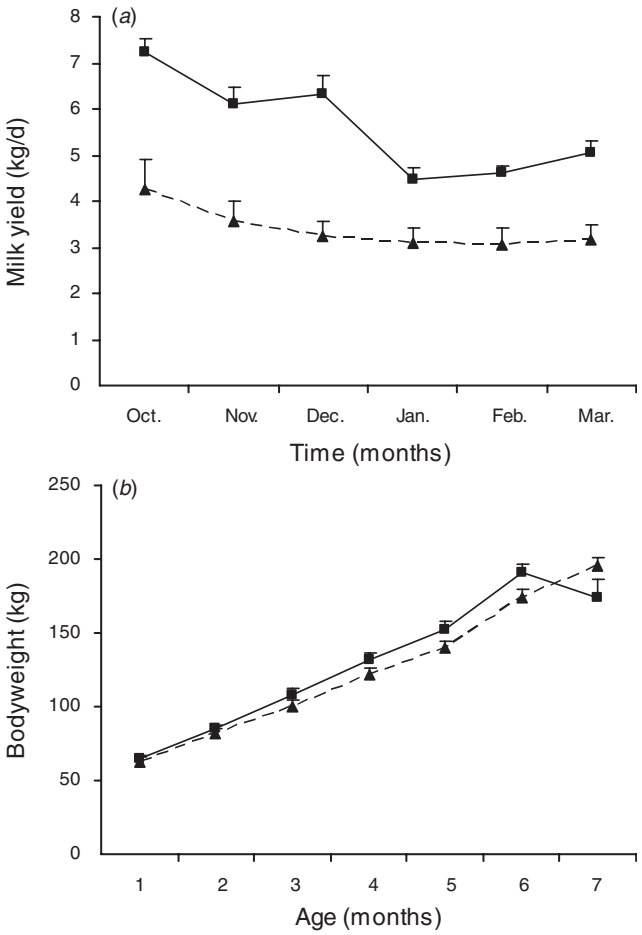

Fig. 1. (*a*) Milk yield during the 6 months before the onset of the experiment in cows with high (■) or low (▲) milk yield (n = 10 per treatment) and (*b*) bodyweight of calves from mothers with high (■) or low (▲) milk yield, before weaning (ages 1–6 months) and after weaning (age 7 months). Data are the mean ± s.e.m.

Table 2. List of behaviours observed and respective descriptions

Behaviour	Description
	Position
Standing	Maintaining an upright position on extended legs
Lying	Lying down in any resting position
	Behaviour
Suckling	Sucking cow's teas and deglutition signals (only present before the nose-flap insertion)
Suckling attempt	Non-rewarded suckling attempt: calves observed to attempt to nuzzle the udder but cannot obtain milk (only present after the nose-flap insertion)
Walking	All four legs are moved with head raised
Vocalisations	Making sounds through the mouth
Grazing	Picking or consuming pasture, with the head above ground, still or moving slowly
Ruminating	Chewing regurgitated boluses of feed
Playing	Activities such as running, jumping, buckling

Distance from the dam

The distance between the cow and the calf was estimated using the cow's body length (BL) as a reference, and recorded at each sampling. Distances considered were 0–1 BL, 1–3 BL, 3–6 BL, or >6 BL.

Data analysis

Milk yield and bodyweight were compared with an ANOVA for repeated-measurements. Average daily gain was compared with ANOVA. The mean frequency of suckling of HMY and LMY calves before nose-flaps were inserted was compared with ANOVA. Frequencies for each behaviour and distances between the mother and the calf are expressed as percentages of total observations, and presented as mean ± s.e.m., and were compared with a mixed model for repeated measurements considering the treatment (HMY or LMY), and the time (day), as well as the interaction between treatment and time as fixed effects, and the calf into each group as a random effect. Post hoc comparisons were done with least significant difference. Differences were considered significant at $\alpha = 0.05$.

Results

Bodyweight

Bodyweight profiles for HMY and LMY calves were similar until the beginning of the experiment (Fig. 1*b*). Whereas HMY calves lost weight after nursing was prevented, LMY gained weight during the same period (ADG from Day –8 to Day 11: –0.9 ± 0.6 *v.* 1.2 ± 0.3 kg/day for HMY and LMY calves, respectively; $P = 0.007$).

Behaviours

Suckling frequency before nursing was prevented was similar for HMY (2.5 ± 0.3%) and LMY (2.5 ± 0.4%) calves. There were no differences between the treatments and no interactions between the day and the treatments in any of the behaviours recorded. However, there was a significant ($P < 0.001$) effect of time in all recorded behaviours, except playing ($P = 0.068$). Means ± s.e.m. and *P*-values for all behaviours are presented in Table 3.

Standing

The frequency of observations in which calves were standing (Fig. 2*a*) decreased on Day 1, increased on Day 2, decreased again on Days 3 and 4, and returned to the initial frequency on Day 5.

Lying

The frequency of observations in which calves were lying was lower on Days 1 and 2 than on Days 3 and 4, and decreased again on Day 5 (Fig. 2*b*).

Walking

The frequency in which calves walked increased on Days 1 and 2, decreased on Day 3, and decreased again on

Table 3. Effect of treatment, day and interactions on the behaviour of calves reared by mothers with high (HMY) or low (LMY) milk yield, after the nose-flap insertion

Values for treatments are the mean percentage of observations ± s.e.m.

Behaviour	Treatment		*P*-value		
	HMY	LMY	Treatment	Day	Treatment × day
Standing	78.0 ± 1.7	80.0 ± 1.6	0.53n.s.	0.0004	0.31n.s.
Lying	7.2 ± 0.5	6.6 ± 0.5	0.55n.s.	0.004	0.27n.s.
Walking	4.2 ± 0.6	4.4 ± 0.5	0.85n.s.	<0.0001	0.91n.s.
Vocalisations	1.7 ± 0.4	2.5 ± 0.7	0.46n.s.	0.0004	0.87n.s.
Suckling attempts	0.8 ± 0.2	1.0 ± 0.3	0.51n.s.	<0.0001	0.80n.s.
Grazing	47.9 ± 2.9	49.0 ± 2.8	0.63n.s.	<0.0001	0.67n.s.
Ruminating	12.4 ± 1.2	11.7 ± 1.0	0.76n.s.	<0.0001	0.36n.s.
Playing	0.11 ± 0.06	0.09 ± 0.06	0.65n.s.	0.06	0.94n.s.

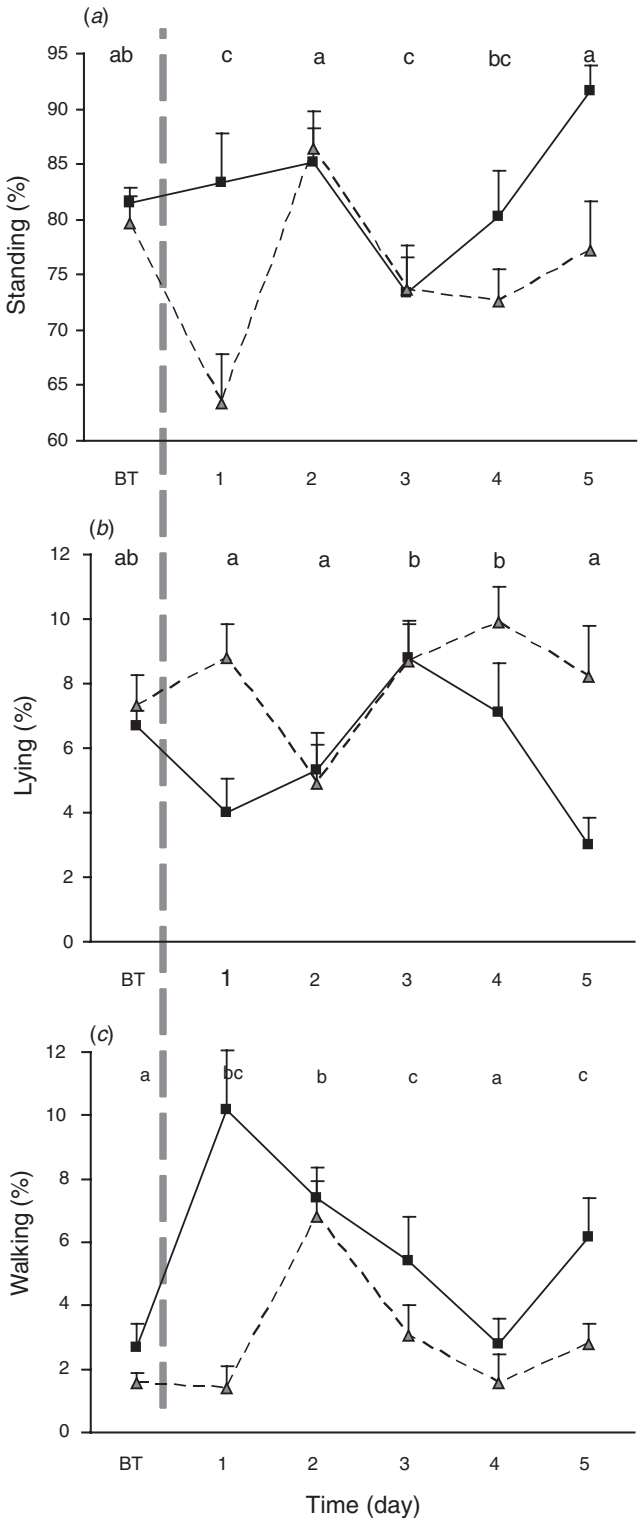

Fig. 2. Frequency of (*a*) standing, (*b*) lying and (*c*) walking in calves reared by cows with high (squares, solid lines) or low (triangles, dashed lines) milk yield. Data are the mean frequency ± s.e.m. of all calves. Vertical dashed line shows when the nose-flaps were inserted. BT = mean data recorded before the insertion of nose-flaps. Time refers to days after insertion of nose-flaps. There were no treatment effects. Different letters indicate significant differences (at $P = 0.05$) between the days.

Day 4, reaching frequencies similar to those observed before nose-flaps were placed, and increased again on Day 5 (Fig. 2*c*).

Suckling attempt

The frequency of calves trying to suckle increased after fitting the nose-flaps (Day 1), and decreased on Days 2 and 3, although it remained greater than those observed before nursing was prevented (Fig. 3*b*). On Days 4 and 5, frequencies were similar to those observed before nursing was prevented.

Vocalisations

The frequency of calves vocalising had a sharp increase on Day 2, decreasing quickly to original frequencies on Day 3, and remaining at those values from then on (Fig. 3*a*).

Grazing

The frequency of observations in which calves were grazing, which is presented in Fig. 3*c*, decreased dramatically the day after nose-flaps were placed, increased on Day 2, and reached frequencies similar to those observed before nose-flap application on Day 3. On Day 5, frequencies were greater than those observed before nose-flaps were placed.

Ruminating

Rumination decreased in the first 2 days after nose-flaps were fitted, increased to original frequencies on Days 3 and 4, and decreased again on Day 5, although to frequencies higher than those observed on Days 1 and 2 (Fig. 3*d*).

Playing

Playing behaviour tended to be affected only by time ($P = 0.07$), and was not affected by treatment.

Distances from the calf to the dam

Distances were affected by cessation of suckling but not by treatment. Overall, after nursing was prevented, the distances between the dams and the calves decreased (Fig. 4). The frequency of observations in which the calves were less than 1 BL away from the dams varied significantly ($P < 0.0001$), increasing significantly on Day 1, and again on Day 2, remaining higher than before nose-flaps were placed until the end of the experiment. Similarly, the frequency of recordings in which calves were between 1 and 3 BL from the dam increased ($P < 0.0001$) on Day 1, and again on Day 2, remaining high on Days 3 and 4. There were no changes in the frequency in which calves were observed between 3 and 6 BL from the dam, but the frequency in which they were farther than 6 BL decreased on Day 1, and again on Day 2, and, although increasing again, remained higher than before nursing was prevented until the end of the experiment.

Discussion

Behavioural responses of calves to the insertion of the nose-flaps – and thus, to cessation of suckling and milk ingestion – were not affected by cows' milk yield. However, during the first days after insertion of the nose-flaps that prevented suckling, all calves

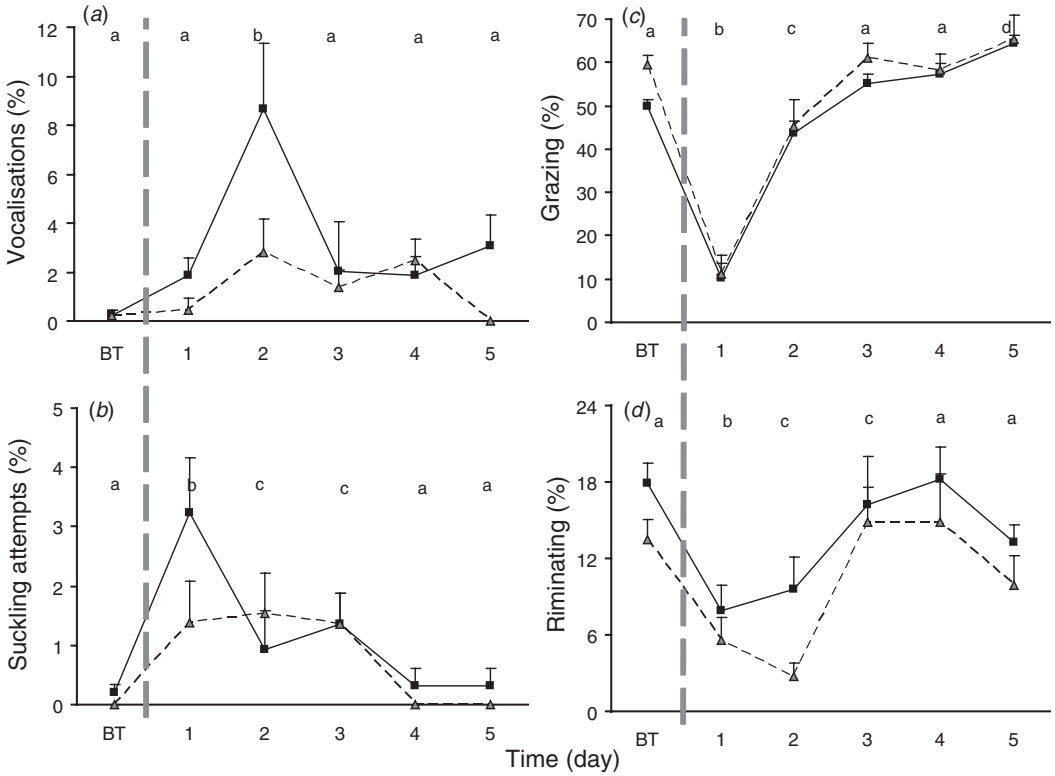

Fig. 3. Frequency of (*a*) vocalisations, (*b*) suckling attempts, (*c*) grazing and (*d*) ruminating in calves reared by cows with high (squares, solid lines) or low (triangles, dashed lines) milk yield. Data are the mean frequency ± s.e.m. of all calves. Vertical dashed line shows when nose-flaps were inserted. BT = mean data recorded before the insertion of nose-flaps. Time refers to days after insertion of the nose-flaps. There were no treatment effects. Different letters indicate significant differences (at $P = 0.05$) between the days.

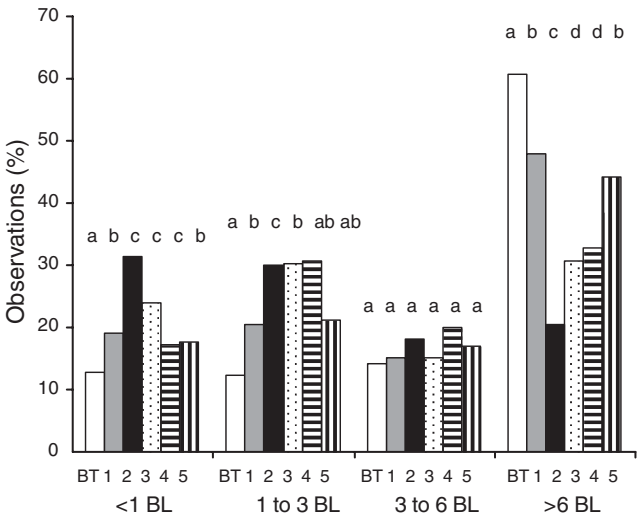

Fig. 4. Frequency of observations of distance between the calf and its dam, estimated as 0 to >6 times the cow's body length. BT = before the insertion of the nose-flaps. BL = body length. Different letters indicate significant differences (at $P = 0.05$) within each category of distance.

displayed a sharp drop in grazing and ruminating, an increase in walking and vocalising, and in the time spent in close proximity to the dam, attempting to suckle – repeatedly butting and nosing the cows' udder. Thus, although this indicates that a loss of milk or cessation of suckling contributes to the weaning distress of 6-month-old calves, we cannot conclude that milk yield of the dam influences this response at this age.

The present results allow us to further discuss the differences in behavioural response to abrupt weaning observed by Ungerfeld *et al*. (2009). In that study, calves reared by high milk-yielding cows appeared more distressed at weaning than were calves reared by low milk-yielding cows. Differences in milk yield between the high and low milk-yielding cows were similar in both studies but, unlike in the present study, where bodyweight of the groups was similar, in the previous study it was 30% higher in the calves reared by higher milk-yielding cows. Thus, the differences found in the previous study in the behaviour of calves reared by cows of different milk yields are possibly best explained by differences in the calves' developmental stage, such as rumen or overall physical development. Alternatively, it is possible that the contact with the mother, which was maintained in the present but not in the previous study, may have buffered possible differences related to cows' milk yield.

From birth until the beginning of the study, the calf liveweight did not differ between the groups; thus, either milk intake did not differ between the groups or calves from lower-producing cows could compensate the lower energy obtained from milk by grazing more efficiently (Ansotegui *et al.* 1991; Mendonça *et al.* 2002). Also, the interruption of suckling was followed by a loss of

weight in calves reared by higher-yielding cows, and a weight gain in those reared by lower-yielding cows. This suggests that the first group experienced a greater loss of energy from milk, which at this age seems to contribute to ~25% of the metabolisable energy (Le Neindre *et al.* 1976; Enríquez 2009). Different reports have attributed changes in the calf ADG to milk intake as varying from 34% (Grings *et al.* 2008) to 50% (Arthur *et al.* 1997), possibly depending on factors such as sex of the calf, season and pasture quality. Although suckling frequency did not differ between the groups before the treatment, it must be noted that this is not considered a good predictor of milk intake (Cameron 1998); thus, there may have been differences in milk intake between the groups. In that case, calves reared by low milk-yielding cows may have compensated for the lower energy intake from milk by grazing more or more efficiently. A further study assessing milk and grass intake could clarify this matter.

The frequency of suckling before the introduction of the nose-flap was ~2.5% of observations in both groups, similar to that reported by Lidfors and Jensen (1988), and did not differ between the treatments. The first day after the nose-flaps were inserted, calves were observed attempting to suckle ~2.5% of the time, and there was a sharp drop in the frequency of grazing and ruminating, responses similar to those reported by Enríquez *et al*. (2010). The reduction in grazing may be associated with physiological or emotional changes triggered by the interruption of suckling. Although it may be argued that the device may have interfered with the ability of calves to graze normally, this effect alone does not seem to explain the reduction in grazing, as calves had undergone this procedure six times on a monthly basis for measurement of cow's milk and, moreover, grazing returned to baseline levels after just 24 h. On Days 2 and 3, there was a rise in some behaviours indicative of distress, suggesting that the behavioural distress response might have been triggered by the maintenance of the devices for more than 24 h, adding to the emotional distress and hunger of calves caused by the effects of low grazing for 24 h. Behaviours indicative of distress included a marked increase in the frequency of vocalisations on Day 2, and an increase in activity on Days 2 and 3. The last was followed by an increase in resting behaviour on Day 4, a response similar to that observed in previous experiments (Quintans *et al.* 2008; Enríquez *et al.* 2010). Perhaps the most noticeable and longer-lasting response to interruption of suckling was the change in distance between the dam and the calf, which also peaked on Day 2 and changed from ~60% of the time spent at more than 6 BL on Day 0 to less than 30% on Day 2 and ~40% on Day 5. Similar results have been reported by other authors (Haley *et al.* 2005; Enríquez *et al.* 2010). As argued by Enríquez *et al*. (2010), if both milk and thermo-tactile stimuli are involved in the mediation of affiliative behaviours in the young mammal (Nelson and Panksepp 1998), the physical closeness with the dam may reflect a need to adjust to physiological changes associated with the sudden loss of milk.

The distress response in calves that maintained social contact with the dam but were suddenly prevented from nursing may have been mediated by hormones and neurotransmitters released in association with milk ingestion or with suckling. For example, hormones involved in the maintenance of social attachment, such as oxytocin, norepinephrine, vasopressin and endogenous opioids (Nelson and Panksepp 1998; Lim and Young 2006), some of which have been shown to be released in response to suckling in calves (Lupoli *et al.* 2001), may mediate the response to sudden cessation of suckling in calves, as has been shown in rat pups (Insel and Winslow 1991; Winslow *et al.* 2000). Also, cholecystokinin, a neurotransmitter released in response to non-nutritive and nutritive suckling in calves (de Passillé *et al.* 1993; Lupoli *et al.* 2001), mediates distress vocalisations induced by deprivation of milk, but not by separation from the dam, as also shown in rat pups (Weller and Gispan 2000). It is possible that the physiological mechanisms that underlie the distress response observed in calves at weaning may not be sensitive to quantitative changes in milk ingestion or suckling, explaining why the sudden and total removal elicited a similar response in both treatments.

In conclusion, the behavioural response to prevention of suckling did not differ between the 6-month-old calves reared by cows with high milk yields and those reared by cows with low milk yields, within the ranges studied. The behavioural response of calves that were prevented from suckling but were kept with the dams indicates that the cessation of suckling contributes to the weaning distress in 6-month-old beef calves.

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