Stilwell, G., Lima, M.S. and Broom, D.M. 2007. Comparing the effect of three different disbudding methods on behaviour and plasma cortisol of calves. *Revista Portuguesa de Ciências Veterinárias,* 102, 281-288.

Comparing the effect of three different disbudding methods on behaviour and plasma cortisol of calves.

Comparação do efeito de três métodos de descorna sobre o comportamento e o cortisol plasmatico de vitelas.

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Summary:
This paper compares the effects of three disbudding methods on cortisol and behaviour of calves. We compared the results of several studies in which thirty six calves were disbudded in the following way: five scoop disbudded (S); seven hot-iron disbudded (HI), eight caustic paste disbudded (CP); eight sham-disbudded with iron (ND-HI); and eight sham-disbudded with paste (ND-CP). Cortisol was measured 15 minutes before the procedures and 1, 3, 6 and 24 hours after dehorning. Behaviour was assessed during the procedure, 15 minutes after dehorning and before each blood sampling. During the procedure HI showed more struggling behaviours than all other groups. Group S struggled more than CP, ND-HI and ND-CP. There was no difference in cortisol base-line and 24 hours values between any of the groups. Compared with all other groups, S had higher cortisol at 1, 3 and 6 hours. At 1 hour cortisol was higher in CP and HI compared with sham-disbudded and in CP compared with HI. Compared with sham-disbudded groups, S showed more pain-related behaviours at all times except 24 hours and more pain behaviours at 6 hours compared with the other methods; CP and HI showed more signs than sham-disbudded until 3 hours.
Although there were differences in age between groups, these results suggest that: scoop-disbudding causes more pain than any other method until 6 hours; dehorning with hot-iron elicits more struggling during the procedure; hot-iron and caustic paste disbudding causes pain until 3 hours but there are no significant differences between them.

Resumo:
Este artigo compara o efeito de três métodos de descorna sobre o cortisol e comportamento de vitelas.

Comparámos os resultados de diversos ensaios em que 36 vitelas foram descornadas da seguinte forma: guilhotina (S, n=5); ferro quente (HI, n=7); pasta caustica (CP, n=8); descorna simulada com ferro (ND-HI, n=8); descorna simulada com pasta (ND-CP, n=8). O cortisol foi medido 15 minutos antes da descorna e à 1, 3, 6 e 24 horas pós-descorna. O comportamento foi avaliado durante a descorna, aos 15 minutos pós-descorna e antes de cada colheita de sangue.
Durante o procedimento HI debateu-se mais do que qualquer outro grupo e S debateu-se mais do que CP, ND-CP, ND-HI.
Não houve diferença no cortisol entre grupos antes da descorna e às 24 horas. À 1, 3 e 6 horas S apresentou mais cortisol do que qualquer outro grupo. Uma hora após descorna CP e HI mostraram cortisol mais elevado do que os grupos simulados e CP tinha níveis mais elevados do HI.
Comparando com os grupos simulados, S apresentou sempre mais sinais de dor excepto às 24 horas e mais às 6 horas quando comparado com os outros métodos. À 1 e 3 horas CP e HI apresentaram mais comportamentos de dor do que ND-CP e ND-HI.
Apesar de existir diferenças de idade entre grupos estes resultados sugerem que a guilhotina causa dor intensa até 6 horas; o ferro quente provoca dor mais intensa durante o procedimento; a descorna por ferro quente ou pasta causa dor até 3 horas, não existindo diferenças comportamentais entre estes métodos.

Introduction

Cattle disbudding is a routine procedure that is considered necessary and acceptable in modern dairy farms (Stafford e Mellor, 2005; EFSA, 2006).
Keeping adult horned cows in free stall dairy farms threatens animal and human safety and welfare because these animals have more potential to cause injury. This is evident by the wounds and various injuries, especially problematic when the udder is affected, that are seen in dairy farms where horned animals are kept.
The International Association for the Study of Pain (IASP) definition for pain is that it is "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage" (Merskey, 1979), modified

slightly by Broom and Fraser (2007) to "an aversive sensation and feeling associated with actual or potential tissue damage". It is also useful to follow the rule by which "animals should be given the benefit of any doubt so that, where there is a choice, judgments should overestimate the intensity of pain to avoid missing animals in pain" (Molony and Kent, 1997). According to these statements, we should consider horn bud amputation or burning, by caustics or heat, as a potentially painful procedure.

Pain management is sometimes ignored or forgotten in farm animals, as it is evident in several surveys in which practitioners report their efforts to control pain after painful procedures (Whay and Huxley, 2005; Huxley and Whay, 2006; Stilwell, unpublished).

Although common sense and legislation (Decreto-Lei nº48/2001) require cornual nerve blocking before disbudding and it is well established that this simple intervention is known to reduce struggling, procedure length and appetite reduction, it is rarely performed in dairy farms. A recent survey with practitioners at the 2007 Congress of the Portuguese Buiatric Association, showed that anaesthesia and analgesia are seldom performed for calf disbudding (Stilwell, unpublished). The main reason for this is the idea that the procedure is not painful, or is only temporarily painful. Other reasons may result from the belief that cattle are very resistant to pain, perhaps a consequence of difficulty in detecting pain-related behaviours in these animals, or that the alleviation of pain is too expensive, or that currently available drugs are not efficient (Whay and Huxley, 2005; Huxley and Whay, 2006). Although some studies (Petrie *et al*., 1995; Morisse *et al*., 1995; McMeekan *et al*., 1998; Graf and Senn, 1999; Vickers *et al*., 2005) have looked at the welfare of calves, following the different disbudding methods, only two tried to compare the effects of hot-iron and caustic paste disbudding (Morisse *et al*., 1995; Vickers *et al*., 2005).

Cortisol is a physiological variable frequently used in the assessment of pain because it is well established that stress activates the Hypothalamus-Pituitary-Adrenal (HPA) axis (Stott, 1981; Broom and Johnson, 2000; Matteri *et al*., 2000; Moberg, 2005). A few minutes after a painful experience cortisol is released and is usually kept above baseline while acute pain is felt. Stimuli other than pain (fear, handling, hunger, anxiety, tissue damage and many other environmental effects) can cause an increase in plasma cortisol, so it is important to control these factors and to understand the circadian cycle that is shared by all individuals (Broom, 1988; Harbuz and Lightman, 1992; Mellor *et al*., 2005; Lane, 2006; Stilwell *et al*., 2008a).

Another way to evaluate pain is to assess behaviour modification. This can be done by objectively measuring the incidence of pain-related behaviours that are usually related to the effort of the animal to get rid of the pain source, to speed recovery or to avoid further injury (Broom e Johnson, 2000; Rushen, 2000; Mellor *et al*., 2005). Head shaking, ear flicking, head rubbing and quick transitions from standing to lying have been used to assess pain following disbudding (Morisse *et al*., 1995; Graf and Senn, 1999; Vickers *et al*., 2005; Stilwell, 2008; Stilwell *et al*., 2008b). These measures also have to be dealt with caution because cattle is a species in which concealment of vulnerability and weakness seems to be adaptive (Broom, 2001; Dobromylskyj *et al*., 2005) and because different animals give different responses to pain as a result of diverse and complex factors such as age, breed, previous experiences, temperament etc. With cattle, very subtle signs (e.g. inert lying) may be of crucial importance while others, very important in other species, might be seldom exhibited. Although the extrapolation "if it hurts humans it must hurt animals" is advisable, the same may not be true for the assessment of pain as it may lead to the exclusion of very specific or subtle changes in behaviours that are important for pain evaluation (Stilwell, 2005).

The 2007 practitioners survey (Stilwell, data not published) showed that the disbudding methods used in Portugal are: chemical disbudding with a caustic paste (80 % of farms), thermo-cautery with a hot-iron (60% of farms) and guillotine disbudding, also known as scoop (12% of farms). Some farms use more than one method.

Caustic paste disbudding is done by applying a thin layer of sodium or calcium hydroxide (pH 14) over the horn bud of calves under the age of 6 weeks. The hot-iron disbudding is done by applying for 30 to 60 seconds a specific device, heated to over 600 ºC, over the horn bud of 6 to 8 weeks old calves, burning the growing tissue at its base. The scoop-disbudding is done by closing the sharp jaws of the tool, amputating the horn and tissue at its base, sometimes including the surrounding skin and bone. This method is usually used in older animals, above the age of 10 weeks.

The handling and restraining of the animals also differ according to the method used. Because calves to be paste-disbudded are small, they are easily subdued and forced to lie down. Animals for hot-iron or scoop disbudding have to be firmly restrained because of need to remain still for longer periods.

The objective of this study was to compare cortisol and behaviour of calves disbudded with the three methods used in Portugal (scoop, hot-iron or caustic paste), so as to try and grade them according to their effects on welfare.

Material and methods

Experiment design

Three dairy farms were selected according to the dehorning method used. We tried to replicate the field conditions in a way to conveniently assess the pain and

distress experienced by the calves. Handling technique, operator and calf age were the ones routinely used in each farm. All animals were used to human handling and regular presence in the paddocks. The different studies were done for several days but all disbudding, blood sampling and behaviour recording started at 10 a.m. in similar weather conditions – clear sky and mild temperatures. In each farm, animals to be disbudded or sham-disbudded were randomly selected and an individual number was sprayed on both flanks. Blood sampling into a heparinised Starsted® tube (7 ml) took place approximately 15 minutes before the disbudding and then at 1, 3, 6 and 24 hours after the disbudding. Blood was immediately centrifuged. Plasma was then frozen at minus 20 ºC. Cortisol was assayed in duplicate and measured by a validated solid radioimmunoassay, without extraction, using a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA) at the Faculdade de Medicina Veterinaria in Lisbon.

Behavioural assessment was done during the procedure in which the degree of struggling was graded from 0 = no struggling behaviour recorded, to 5 = all struggling behaviours were recorded. At 15 minutes, 1, 3, 6 and 24 hours after the disbudding the incidence of five different pain-related behaviours were recorded for a period of 15 minutes. The behaviours assessed are described in Table 1.

Handling and blood collection was as quick and peaceful as possible so as to reduce stress and was done by an experienced veterinarian inside the first 30 seconds after restrain

The number of animals in each group and mean age are presented in Table 2.

Each farm's husbandry conditions and disbudding method used were as follows:

Scoop disbudding: this study was done in a single day. Five Holstein-Frisian female calves with mean age 117±32 days were kept in concrete floored paddocks. Concentrate, grass hay and water were permanently available. For disbudding the calves were put into a crunch and the head was restrained with a rope. The scoop-dehorner was pushed against the head and rapidly closed to cut off the horn base. The procedure was repeated for the other bud and took no more than 45 seconds in total. Due to farm constraints, there was no sham scoop-disbudded group but two other groups were formed to test regional anaesthesia and analgesia efficacy (Stilwell, 2008).

Hot-iron disbudding: this study was repeated with different animals along several days. Fifteen female calves with mean age 98±15 days were kept in concrete floored paddocks. They were already weaned and were eating concentrate and alfalfa/grass hay. The disbudding procedure was done with the calves standing and the head restrained by a head-halter. While one person gently pressed the calves against a wall another operator applied the hot-iron to the base of each horn bud for the duration of 30 to 45 seconds. The sham-iron-disbudding was done in the same way but a cold device was applied to the head. The total procedure took from 90 to 120 seconds and was always done by the same stockman.

Caustic-paste disbudding: this study was repeated with different animals along several days. Sixteen Holstein-Friesian female calves with mean age 25±10 days, kept in a large straw-bedded paddock with access to a computer-controlled milk distributor and *ad libitum* concentrate and grass hay. These animals were randomly allocated to treatment groups with no age differences: CP - caustic paste disbudded with no treatment (n=8) and ND-CP - sham-disbudded (n=8).

Table 1 – Description of the behaviours recorded at disbudding and for 24 hours after the procedure

Struggling at dehorning	Description
Hot-iron and Scoop (occurrence of any of the five behaviours were added)	Lifting front limbs; falling on back limbs; backing; vocalization; open mouth.
Caustic paste (occurrence of any of the five behaviours were added).	Trying to rise; shaking head; stretching back limbs; vocalization; open mouth.
Behaviours after dehorning	**Description**
Ear flick	Flicking ears with no apparent reason (e.g. presence of flies).
Head shake	Shaking head.
Head rub	Scratch head against objects or using back leg.
Transitions (lying/raising)	Lying and raising hastily with no resting objective.
Inert lying	Sternal lying with head on flank and ignoring external stimulus.

Table 2 – Number and age (mean ±SD) of calves in each group accordingly to the disbudding method used

	Disbudding method				
	Scoop (S)	Hot-iron (HI)	Caustic Paste (CP)	Sham-dehorned (ND-HI)	Sham-dehorned (ND-CP)
Animals (n)	5	7	8	8	8
Age in days	117 ±33[a]	98 ±15[b]	25 ±10[c]	76 ±11[b]	31 ±5[c]

Different superscript letters indicate difference in age between groups for which $p < 0.05$

For the disbudding, animals were forced to lie down, the hair around the horn bud was clipped and the paste was applied to each horn. Sham-paste-disbudded animals were handled in the same way but an inert gel was applied instead of the paste. The total procedure took no more than 60 seconds.

Two calves were removed from the study (one from group ND-CP and one from group HI) due to respiratory disease and the need for treatment.

Statistical analysis

Distributions of these variables were shown by Levene and Shapiro–Wilks tests to be non-normal, so non-parametric analyses were used. Differences between the five groups at each time were determined by the Mann–Whitney *U*-test following a Kruskal––Wallis one-way analysis of variance. *P*-values less than 0.05 were considered significant. SPSS® for Windows (version 14) was used for the analysis.

Results

There was a difference in ages between the disbudded groups (Table 2) but not between the two hot-iron groups (HI and ND-HI) and the two caustic paste groups (CP and ND-CP).

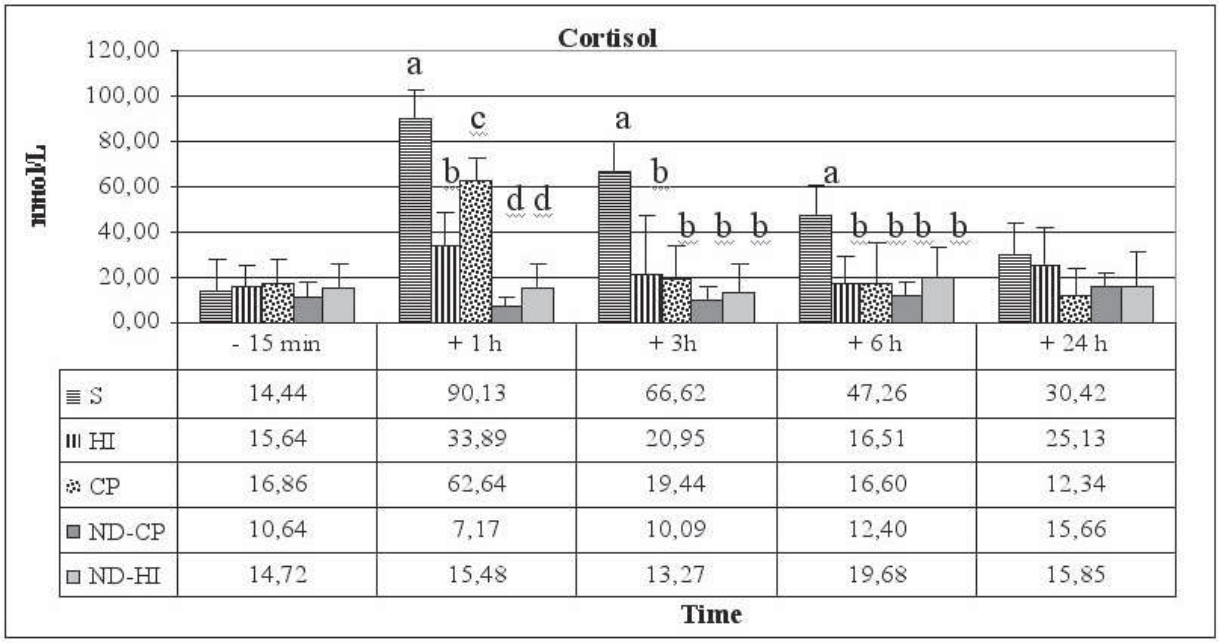
Comparing cortisol values at each moment (Figure 1) we found that there were no differences between groups at –15 minutes (baseline) or at 24 hours (p=0.669 and 0.126, respectively). Additionally, there were no differences in cortisol between the two sham-disbudded groups at any time (always p≥0.05).

In contrast, at 1 hour, both sham-disbudded groups showed lower cortisol than S (p<0.004) and CP calves (p<0.001) and group HI had higher cortisol than ND-HI (p=0.002) but only showed a trend towards difference (p=0.051) when compared with group ND-CP. When comparing the different disbudding methods at 1 hour the S group showed higher cortisol levels compared to CP and HI groups (p=0.019 and p=0.003, respectively), and CP had higher cortisol than HI (p=0.001). At 3 and 6 hours there was still a difference between S and the other disbudded groups: for CP p=0.002 and p=0.019, respectively; and for HI p=0.03 and p=0.01, respectively.

The different pain-related behaviour's incidence for the various groups is shown in Table 3. Struggling was significantly higher in the HI group than in any of the other groups (p<0.01) and group S struggled more than both sham-disbudded and the CP animals (p<0.05). In contrast there were no differences in struggling between sham-disbudded groups and caustic paste disbudded animals (p>0.491).

The S group showed more pain behaviours than sham-disbudded at all times (p<0.001) except at 24 hours but only at 6 hours when compared with the two other disbudded groups (CP p=0.03 and HI p=0.048). The HI group showed more behaviours than the ND-HI at 15 minutes, 1 hour and 3 hours (at 6 hours there was a trend with p=0.054) and more behaviours than ND-CP at all times except at 24 hours. The group CP showed higher incidence of pain-related behaviours when compared to both sham-disbudded groups at 15 minutes and 1 hour and at 3 hours when compared with ND-HI.

Figure 1 – Mean ±SD plasma cortisol of calves disbudded by different methods



S - Scoop; HI - hot-iron; CP - caustic paste; ND-HI – sham-disbudded with iron; ND-CP – sham-disbudded with paste. Different superscript letters in each period of time indicate difference between groups for which $p < 0.05$

Table 3 – Degree of struggling during disbudding and individual and total incidence of pain-related behaviours (mean ±SD) of calves for the first 24 hours post-disbudding with scoop (S), hot-iron (HI), caustic paste (CP) or sham-disbudded (ND-HI and ND-CP)

		Time from disbudding						
Group	Behaviour	0	+ 15 min	+ 1 h	+ 3 h	+ 6 h	+ 24 h	Total
	Struggling (¥)	2,2[a]						
	Head shake		10	16	5	5	4	40
	Ear flick		4	3	1	7	3	18
S	Head rubbing		7	9	3	6	2	27
n=5	Transitions		1	0	0	0	0	1
	Inert lying		0	0	0	0	0	0
	Total		22	28	9	18	9	86
	Mean		**4.40[a]**	**5.60[a]**	**1.80[a]**	**3.60[a]**	**1.80[a]**	**17.2**
	Struggling (¥)	3,43[b]						
	Head shake		9	8	2	4	0	23
	Ear flick		16	16	3	4	3	42
HI	Head rubbing		18	6	5	3	0	32
n=7	Transitions		0	1	0	1	0	2
	Inert lying		0	0	0	1	0	1
	Total		43	31	10	13	3	100
	Mean		**6.14[a]**	**4.43[a]**	**1.43[a]**	**1.86[b#]**	**0.43[a]**	**14.29**
	Struggling (¥)	0,8[c]						
	Head shake		22	8	0	0	0	30
	Ear flick		9	3	6	0	0	18
CP	Head rubbing		13	8	7	2	1	31
n=8	Transitions		7	3	2	2	0	14
	Inert lying		0	1	4	1	0	3
	Total		51	23	19	5	1	99
	Mean		**6.38[a]**	**2.88[a]**	**2.38[a]**	**0.50[bc]**	**0.13[a]**	**12.38**
	Struggling (¥)	1,00[c]						
	Head shake		1	0	1	1	2	5
	Ear flick		3	1	0	2	1	7
ND-HI	Head rubbing		0	0	0	0	0	0
n=8	Transitions		0	0	0	0	0	0
	Inert lying		0	0	0	0	0	0
	Total		4	1	1	3	3	12
	Mean		**0.6[b]**	**0.3[b]**	**0.1[b]**	**0.4[bc]**	**0.4[a]**	**1.7**
	Struggling (¥)	0,80[c]						
	Head shake		0	0	1	0	2	3
	Ear flick		3	0	0	0	1	4
ND-CP	Head rubbing		0	1	0	1	0	2
n=6	Transitions		0	0	1	0	0	1
	Inert lying		0	0	0	0	0	0
	Total		3	1	2	1	3	10
	Mean		**0.5[b]**	**0.2[b]**	**0.3[b]**	**0.2[c]**	**0.5[a]**	**1.7**

(¥) Results from adding the occurrence of five struggling behaviours. From 0=no struggling behaviour observed to 5= all struggling behaviours observed. Different superscript letters in each period of time indicate difference between groups for which $p < 0.05$.

When comparing HI and CP groups no differences were found in the incidence of pain-related behaviours although at 6 hours there was a trend (p=0.054) for higher behaviour incidence in HI than in the CP group.

By comparing the incidence of individual behaviours (Table 3) between the different groups we found that a higher number of very active behaviours (head-shaking and head-rubbing) were seen in groups S and HI, but a more even distribution in group CP. Transitions were very rarely recorded in S and HI animals (one or two observations) but were common in CP group (14 observations). Inert-lying behaviour was recorded in three caustic-paste disbudded animals, in one hot-iron disbudded calf and never seen in the scoop or sham-disbudded groups. Vocalizations were recorded only during the actual disbudding of two animals in the HI group. In the sham-disbudded animals ear-flicking was almost the only behaviour recorded. Although not statistically significant, nine pain-related behaviours were still observed in the smaller S group at 24 hours compared with three observations in the HI and one in the CP group.

Discussion

Although these results were taken from studies at different farms, the weather, time of day, and study design were very similar. There was a difference in the age of the animals, the scoop-disbudded were the oldest and the paste-disbudded the youngest, because the age at which these methods are applicable do not overlap. There were also differences in some husbandry conditions, namely the pens' floor and the feeding (calves in the ND-CP and CP groups were not yet weaned). However, the fact that there were no differences in baseline and '24 hours' cortisol levels between groups and between sham-disbudded groups at all times, suggests that handling, environment and calf's age had no effect on the results. Other studies have compared cortisol and behaviour of calves disbudded at different ages and found no significant differences in the responses (Taschke and Folsch, 1997).

The hot-iron disbudded group showed a very high degree of struggling, average grade of 3.63, and included the only two animals that vocalized in all the studies (both calves with maximum struggling score of 5). These behaviours are a sign of intense distress that was to be expected when an extremely hot device is in contact with live tissue for more than 30 seconds and the animals are forcedly restrained. The fact that relatively few animals vocalize when exposed to intense stress and pain is probably due to cattle bio-adaptation by which signs of vulnerability are hidden from potential predators (Broom, 2001).

The actual disbudding through amputation is probably equally painful but the procedure is very quick and so the animal does not have to struggle so much to get away from restraint and from the aggression source. With caustic-paste, the struggling was minimal and not different from sham-disbudded calves. We suggest that there are two reasons for this: because product activation, caustic activity and, consequently, pain takes a few seconds to come to effect; and because younger animals (~1 month) are more used to handling and usually resist less to restrain and human proximity.

The method that showed the uppermost and longest pain-related distress was the scoop-disbudding. The extent and duration of cortisol increase was similar to that found in other studies (Petrie *et al*., 1995; Stafford *et al*., 2003). The cortisol level was higher in the S group compared with all other groups at all times until, at least, 6 hours after the procedure. The cortisol' response level at 1 hour may even have been limited by the "ceiling effect" that is described as the maximum hormonal level possibly attained after a negative experience (Molony and Kent, 1997; Molony *et al*., 2002). This physiological limitation should also be taken in account when reading the results at 6 hours because the decrease may be due to the exhaustion of the system and not necessarily to the reduction in pain, which was seen to be still very severe by the pain--related behaviour observations.

The other two disbudding methods only showed higher cortisol at 1 hour, when compared with sham--disbudded, suggesting that pain is limited to the first few hours after the procedure. Morisse *et al*. (1995) showed similar results although differences were less evident. There were also important differences between the caustic-paste and hot-iron groups indicating that the first causes a more intense pain at 1 hour. Similar results were found by Morisse *et al*. (1995).

However, the behaviour-incidence analysis show that at 1 and 3 hours all three methods cause similar pain and that at 6 hours the pain is much more severe in the scoop-disbudded than in all the other groups. In contrast with the cortisol results, the comparison of behaviour incidence in the ND-CP and ND-HI groups with the CP or HI ones show that the disbudded calves still suffer some pain at 6 hours and that there are no differences between these two disbudded groups. It is worth mentioning that the S group shows a wavering in the behaviour incidence that is not found in any of the other groups – a decrease at 3 hours compared with 1 hour and a new, very significant, increase at 6 hours. This may have been a consequence of the intensity of pain suffered by these animals that reduced their activity in response to a previous very painful period or because the severe inflammation created a state of hyperalgesia that resulted in extra pain. Another possibility is that the decrease in behaviour at 3 hours was due to an external factor, not evident to the observer, which distracted the calves.

Although there were only five calves scoop-disbudded, at 24 hours there were still nine pain-related behaviours recorded in the S group compared with three for the HI and both ND groups and one for the CP group. This may indicate that pain is felt for long periods after scoop-disbudding. However, the fact that there was a very large variation between individuals meant that this difference was found to be non--significant.

By comparing the total incidence of each behaviour within each group, it was shown that the scoop disbudding caused almost exclusively three behaviours – head-shaking, ear-flicking and head-rubbing, being the first the most prevalent. The majority of behaviours shown by the hot-iron disbudded calves were also these three but the most prevalent was "ear-flicks". In contrast, the behaviours caused by caustic-paste disbudding were more evenly distributed. The animals disbudded by caustic-paste showed a relatively high incidence of "transitions" compared with the other methods but it is not certain if this was due to the difference in the type of pain or the age of the animals. Other studies (Vickers *et al*., 2005) showed a high incidence of "transitions" after hot-iron disbudding in animals aged 10 to 30 days. However, these animals had been sedated a few hours previously

with xylazine and it was not possible to rule out an ongoing response to the sedative.

Very few animals showed the "inert-lying" behaviour and of the four that did show it three were from the CP group. The animals showing "inert lying" behaviour were also the ones that showed cortisol levels higher than the group average. High cortisol levels have been shown to be related to states of depression (Tse, 2004). Lane (2006) also suggests that a state of helplessness and frustration, that may result from a situation in which an animal is not able to escape or cope with pain, causes a state very similar to depression. Very high levels of glucocorticoids have been detected in animals suffering from this kind of frustration (Gregory, 2004; Sumida *et al*., 2004). The inert lying may be the best way these calves have to cope with the pain caused by some methods of disbudding. At this moment we can not say if this is a response to the type of pain (e.g. chemical burn) or the way very young animals react to severe pain, distress and high cortisol. It is well known that young calves adopt an inert posture when frightened or anxious, for example when left unattended in the field by their dam, and so this behaviour may be a biological response to stress, triggered by high levels of cortisol.

Our results show that comparing different methods is useful even when there are differences in age because it shows what really happens in the field. We also showed that by assessing the physiological (cortisol) and behavioural response it is more likely to understand the differences in intensity and duration of pain-related distress. This is especially true for the methods that cause long lasting pain because cortisol is less reliable for dull and chronic pain (Broom and Johnson, 2000; Mellor *et al*., 2005; Lane, 2006). Thus the fact that cortisol returns to baseline levels should not be seen as a sign of "no pain".

We conclude that amputation-disbudding causes more intense and long-lasting pain when compared with caustic-paste and hot-iron disbudding. Morisse *et al*. (1995) and the EFSA report on welfare of calves (2006) assert that caustic paste is more painful than hot-iron disbudding, but Vickers *et al*. (2005) suggest that the contrary is true. Our results show that hot-iron disbudding causes very severe distress during the procedure and pain that lasts for at least 3h. Compared with hot-iron, paste disbudding causes less struggling during the procedure, higher cortisol level at 1 hour and the same incidence of pain-related behaviours until 3 hours. Although more studies should be made to evaluate differences during the first hour, we suggest that the overall distress does not differ between these two methods.

Our study also shows that the behaviours performed by animals in pain differ with the method and, probably, with the age of the animal. This should be taken in account in the field at the risk of considering a painful procedure as non painful. For example, transitions from standing to lying and back to standing seem to be very frequent after paste disbudding but not after other methods of disbudding. We also concluded that young animals react to distress caused by burns by adopting an apathetic attitude that may prevent the recognition of pain in these animals.

Acknowledgements

The authors acknowledge Prof Dr Luísa Mateus; Eng Marta Vacas de Carvalho; Casal de Quintanelas and Alexandre Arriaga e Cunha; Barão & Barão Lda and Eng Ricardo Basílio, Agropecuária Afonso Paisana and Dr João Paisana; Dr Rita Campos de Carvalho.

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