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Effects of hot-iron disbudding, using regional anaesthesia with and without analgesia, on cortisol and behaviour of calves

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Abstract

The objective of this study was to assess cortisol and behaviour changes in calves hot-iron disbudded after different analgesic protocols with carprofen. We assessed the response of calves (n=27) at 1, 3, 6 and 24 hours after disbudding with local anaesthesia (DA), local anaesthesia plus carprofen (DAC), disbudded only (D) or sham-disbudded (ND). Immediately after the procedure, pain-related behaviours were more frequent in D than in any other group. At 1 h cortisol was higher in D compared with all other groups. At 3 h ND showed lower cortisol than all other groups but these did not differ from their own baseline levels. At 1 h D showed more head-shakes, ear-flicks and head-rubs than all other groups.

Groups D (3 h) and DA (3 and 6 h) showed more ear-flicks and head-rubs compared with DAC and ND. Head-rubbing, head-shaking and ear-flicking are useful behaviours for evaluating pain but quick transition from standing to lying is not a reliable behaviour to assess pain after hot-iron disbudding. In conclusion, hot-iron disbudding causes pain in calves for at least 3 hours and only the association of local anaesthesia with carprofen efficiently controls pain for 24 hours.

Keywords: calf disbudding; pain; plasma cortisol; behaviour; welfare

Introduction

Disbudding young female dairy calves is a routine procedure in most dairy farms. The objective is to reduce injury to other animals and humans caused by horned cows. The most common methods of destroying the growing horn tissue are: heat-cauterization, by applying on the horn base a device heated to above 600ºC for ~30 seconds (thermal or hot-iron disbudding), and chemical-burning with sodium hydroxide paste (caustic disbudding). Thermal disbudding leads to the destruction of all the epidermal and dermal layers extending down to the subcutaneous tissue, but it may also cause tissue damage and oedema that extends beyond the burn-site increasing the sensitized area (Junger *et al*, 2002).

Pain-related distress can be assessed by measuring physiological and behavioural changes in animals submitted to the procedure compared with sham-disbudded ones (Morton *et al*, 1985; Molony and Kent, 1997; Mellor *et al*, 2005). Cortisol has been shown to be accurate to assess the occurrence of pain after hot-iron dehorning because the hypothalamic-pituitary-adrenal cortex axis is activated during the distress caused by the

52 procedure (Petrie *et al*, 1995; Graf and Senn, 1999; Doherty *et al*, 2007). Pain-related
53 behaviours recorded after disbudding have been: head-shaking, ear-flicking, rapid transition
54 from standing to lying and to standing again, and rubbing the head with the hind feet
55 (Morrise *et al*, 1995; Grøndahl-Nielsen *et al*, 1999; Doherty *et al*, 2007).

56 Local anaesthetic drugs act by inhibiting sodium-channels to impede nerve
57 depolarization and conduction. Lidocaine 2% is the most commonly used local anaesthetic
58 in cattle practice and its blocking effect persists for 60 to 90 minutes after injection (Muir *et*
59 *al*, 1995; Anderson and Muir, 2005).

60 Carprofen is a NSAID with a mode of action that is not entirely known, although it
61 is considered to be a relatively poor cyclo-oxygenase inhibitor. However, it has shown
62 analgesic properties similar to opioids after surgery (Nolan, 2005). Several studies on cats
63 (Al-Gizawiy *et al*, 2004), dogs (Lascelles *et al*, 1998), horses (Johnson *et al*, 1993) and
64 cattle Stilwell *et al*, 2008a) reveal that it is an excellent post-surgical analgesic. The half-
65 life of carprofen depends on the species, but has been established to be > 34 hours in 17-
66 week-old calves and 44 to 64 hours in adult cows (Delatour *et al*, 1996; Lees *et al*, 1996). A
67 long-lasting anti-inflammatory effect of carprofen has been found for cattle (Balmer *et al*,
68 1997). The duration of the analgesic effect of carprofen in calves has not been established
69 although it has been shown to reduce cortisol and pain-related behaviours for 48 hours in
70 clamp-castrated calves (Stilwell *et al*, 2008a). Carprofen is a drug approved for cattle in
71 most of Europe and is currently used by many bovine practitioners.

72 Other non-steroidal-anti-inflammatory drugs (ketoprofen, meloxicam and flunixin-
73 meglumine) have been studied in calves after disbudding.

74 The objectives of this study were to assess pain-related distress in calves after hot-
75 iron disbudding by measuring physiological (plasma cortisol concentration) and

behavioural responses after different analgesic protocols with carprofen and lidocaine local nerve block.

Material and Methods

Farm and animals

All the experiments were done at the same 300 cow dairy farm. At this farm new-born calves are kept in individual hutches, bedded with straw, until weaning. Before weaning they are fed milk at 5% of body-weight in the morning and evening and have free access to grass hay, 18% protein calf starter and water. Weaning is done when the calf eats over half a kilogram of concentrate per day for three consecutive days. After weaning calves are moved to an open stable and have free access to concentrate, grass and alfalfa hay and water.

Experimental Procedures and Design

In this study the effects of the routine procedure of hot-iron disbudding was investigated for the first 24 hours.

The "Centro de Investigação Interdisciplinar em Sanidade Animal" (CIISA) Committee for post-doc studies, of the Lisbon Faculdade de Medicina Veterinaria, approved all animal use in this project. The disbudding protocol was the same as that usually carried out at the farm.

Common procedures

Disbudding was carried out between 10 and 11 a.m. during several days by the same operator, blind to the treatments. The iron was electrically-heated and applied over the horn

100 bud for ~30 seconds for each horn, producing a deep burn of the tissue at the base of the
101 horn. A cold device was applied for the same time to the control calves (sham-disbudded).
102 Disbudding was done in groups of 4 to 6 animals a few days after weaning, corresponding
103 to the age of 8 to 10 weeks.

104 Cornual nerve anaesthesia was achieved by the injection of 5 ml of 2% lidocaine
105 (Anestesin ®, Laboratorio Sorologico, Portugal), without adrenaline, just ventral to the
106 lateral edge of the frontal bone, midway from the base of the horn to the lateral cantus of
107 the eye (Noordsy, 1994; Greene, 2003). In control groups groups, a 0.9% saline solution
108 was administered in the same way. Carprofen (2 ml, approx 1.4 mg/kg; Rimadyl®, Pfizer-
109 Animal Health, Dundee, UK) was given i.v. 15 minutes before the procedure was carried
110 out or, in controls, the same dose of a saline solution was given i.v. Animals' approximate
111 weight was estimated by body size.

112 Blood sampling (7ml) into a heparinised tube was by left jugular venipuncture.
113 Blood was kept on ice then centrifuged and the plasma frozen (-20C). Cortisol was assayed
114 in duplicate and measured by a validated solid radioimmunoassay, without extraction, using
115 a commercial kit (Coat-A-Count; Diagnostic Product Corporation, Los Angeles, CA, USA)
116 at the Faculdade de Medicina Veterinaria. The lowest detectable concentration of cortisol
117 was 1.0 nmol/l. The inter-assay coefficients of variation was 6.5% for 1 ng/mL and 3,4%
118 for 5 ng/mL and the intra-assay coefficients of variation was 5.6% (Multivalent Control
119 Module, DPC, Los Angeles, CA, USA) (Rodbard, 1974).

120 Behaviour was assessed by an experienced veterinarian blind to the treatments. The
121 frequencies of four pain-related behaviours (ear-flicking, head-shaking, head rubbing with
122 hind foot and quick transitions from standing to lying and back to standing) were recorded
123 by a veterinarian just before each blood sampling. The total behaviour incidence (sum of all

124 behaviours within each group divided by the number of animals in that group) was also
125 calculated.
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127
128 *Animals*
129 Twenty-eight female calves, mean age 88 ±17 days, were randomly assigned to four
130 groups: DA: disbudded after lidocaine injection (n=7); DAC: disbudded after lidocaine and
131 carprofen injection (n=7); D: disbudded after treatments with saline (n=7); ND: sham-
132 disbudded after treatments with saline (n=8). Blood was collected 15 min before the
133 procedure and then at 1, 3, 6 and 24 hours after disbudding. Behaviour was assessed for
134 periods of 15 minutes at 15 min, 1, 3, 6 and 24 hours after disbudding.
135 One calf was eliminated from the DA group because of clinical disease signs shown
136 during the experiment.
137
138 *Statistical analysis*
139
140 The between-day differences for plasma cortisol concentrations and behaviour incidence
141 within groups were not significant so data were pooled.
142 The plasma cortisol analysis of variance was done with the PROC MIXED from SAS
143 (SAS, 2004) using the following mixed linear model: $Y = X\beta + Z\gamma + \varepsilon$, that includes the
144 treatment and time and their respective interaction as fixed effects and the calves as the
145 unknown random effect. In addition the Least Squares Means for each treatment*time

combination was calculated as well as the differences between the means and the respective t-test.

Distributions of the behaviour variables were shown by Levene and Shapiro-Wilkes tests to be non-normal, so non-parametric analyses were used. Behaviour means and standard errors were calculated using PROC GENMOD from SAS (SAS, 2004) with a model that included the effect of treatment and time and their respective interaction. The incidence of the four pain-related behaviours were then analyzed with the PROC NPAR1WAY from SAS (SAS, 2004), using Wilcoxon and Median tests to compare each pair of treatments (2x2) at different times.

For all tests differences for which $p<0.05$ were considered significant.

Results

There were no differences in age between groups. DA (83 ±15); DAC (96 ±20); D (98 ±15); ND (76 ±11).

No differences were found between groups base-line plasma cortisol concentrations levels (Table 1). At 1 hour calves disbudded with no treatment showed higher cortisol than sham-disbudded and calves treated with regional anaesthesia and carprofen ($P<0.01$). At 3 hours calves treated only with lidocaine (DA) showed higher cortisol than sham-disbudded calves ($P<0.05$). Only D group showed differences when compared with baseline and only at 1 hour after the procedure.

There were no differences in transitions behaviour between groups at any time (Figure 1).

The other three behaviours' incidences are shown in Table 2-4. Immediately after the procedure, D and DA showed significantly more head shakes than DAC and ND but D

showed more ear flicks and head rubs than all the other groups. One hour after disbudding D calves showed more head shakes and ear flicks than all other groups and more head rubs than DAC and ND. Calves treated with local anaesthesia showed more ear flicks (1, 3 nd 6 h) and head rubs (3 and 6 h) than sham-dehorned animals. At no time did DAC showed any difference when compared with ND animals.

When assessing the total behaviour incidence (Table 5), D group shows more behaviours than D, DAC and ND (15 min); than DAC and ND (1 h); and than ND (3 h). Animals disbudded only with local anaesthesia show more signs at 15 min, 1, 3 and 6 hours when compared with DC and ND. Along time the disbudded-only animals showed a higher incidence of behaviours at 10 min than at all other times ($P<0.05$) and more behaviours at 1 hour than at 3, 6 or 24 ($P<0.05$). The group disbudded with lidocaine alone showed more behaviours at 10 min, 1 h and 3 hours when compared with the incidence at 24 hours ($P<0.05$).

DISCUSSION

Several studies have confirmed that hot-iron disbudding causes pain in calves for at least 2 hours (Petrie *et al*, 1995; Morisse *et al*, 1995; Doherty *et al*, 2007). The cortisol results of our Experiment 1 show that distress is present at 1 hour but no difference is evident at 3 hours when compared with sham-disbudded animals. However behaviour analysis shows a high incidence of altered behaviours at 3 hours, suggesting that, although not causing a noticeable rise in plasma cortisol, discomfort is present for longer than previously assumed. Although some studies with rats show that mechanical hyperalgesia is still present 2 weeks after full-thickness thermal burns (Summer *et al*, 2007), we did not find any evidence of pain-related distress in disbudded calves at 6 or 24 hours. This could

194 be due to species differences, a relatively smaller burned area or because we did not look at
195 measures that effectively assess hyperalgesia and chronic pain.

196 Some studies have been contradictory as to the efficacy of regional anaesthesia.
197 Petrie *et al* (1995), using 2% lidocaine, and Doherty *et al* (2007), using 2% and 5%
198 lidocaine, concluded that regional anaesthesia is not very efficacious. In contrast, Grøndahl-
199 Nielsen *et al* (1999) and Graf and Senn (1999) showed that cornual nerve block markedly
200 attenuates behavioural and physiological response for the first two hours after the
201 procedure. However all studies that looked at the struggling during the procedure agree that
202 cornual nerve blocking is efficient in reducing signs of pain. Our results also show a
203 positive effect by reducing the degree of struggling compared with animals disbudded with
204 no anaesthesia. The Experiment 1 cortisol results indicate that regional anaesthesia is
205 efficient in controlling pain for 24 hours, but analysing the pain-related behaviour incidence
206 we show that pain is present as early as 1 hour and for 3 hours after disbudding. Cortisol
207 levels in Experiment 2 also show that animals treated with regional anaesthesia suffer some
208 distress immediately after the procedure when compared with sham-disbudded, although to
209 a smaller degree than control disbudded ones. This could be due to the handling during
210 disbudding but the fact that the sham-disbudded calves (submitted to the same handling)
211 did not show an increase suggests that some pain is felt even when regional anaesthesia is
212 given.

213 The results from blood collected after the nerve block supposedly had subsided
214 (Experiment 3) show a rise in cortisol in all treated calves although different calves
215 responded at different times (data not shown). This is probably why no difference between
216 groups is apparent at 90 and 120 min in Experiment 3 or at 3 hours in Experiment 1, when
217 comparing disbudded with sham-disbudded animals, but for overall cortisol response

between the two groups in Experiment 3, the difference was evident. Graf and Senn (1999) and Grøndahl-Nielsen *et al* (1999) also showed a delayed increase in cortisol of lidocaine-treated animals at 180 and 210 min post-disbudding. Doherty *et al* (2007) did find a similar increase at 4 hours after blocking with 5% lidocaine but not when using 2% lidocaine. In contrast, behaviour differences are evident at 90 and 120 minutes (Experiment 3) and at 3 hours (Experiment 1) after disbudding suggesting that pain, probably due to extensive inflammation that follows deep thermal burns (Junger *et al*, 2002) is felt by calves when regional anaesthesia subsides. These results also show that the duration of nerve block varies between individuals, perhaps due to anatomical or physiological differences.

The use of both regional anaesthesia and a non-steroidal-anti-inflammatory drug (NSAID) is shown here to efficiently control pain-related distress after hot-iron disbudding. All previous studies using NSAID have used ketoprofen as the analgesic (McMeekan *et al,* 1998; Faulkner and Weary, 2000; Milligan *et al*, 2004). With this study we demonstrated that regional anaesthesia together with carprofen is equally efficient in reducing or eliminating the rise in plasma cortisol and pain-related behaviours from immediately after disbudding to 24 hours after the procedure.

Only two studies have looked at the effect of a NSAID given alone and pre-emptively: McMeekan *et al* (1998) showed that ketoprofen alone had no effect in controlling pain after scoop-dehorning and Stilwell *et al* (2008b) found the same result when using flunixin-meglumine after paste disbudding. In the present study we showed that carprofen, without regional analgesia, only reduces the intensity of the cortisol and behaviour response at 30 minutes after disbudding when compared with disbudded control animals. Carprofen alone also showed a trend towards the reduction of struggling during the procedure compared with non-treated animals. These results suggest that carprofen

242 alone does have an analgesic effect but not sufficient to eliminate pain caused by hot-iron
243 disbudding.

244 We conclude that hot-iron disbudding of young calves is a procedure that causes
245 severe pain during the procedure and for, at least, 3 hours. Regional anaesthesia is efficient
246 in reducing struggling and pain signs for the first hour but does not prevent pain-distress
247 when nerve blocking subsides. Carprofen given alone and pre-emptively does not reduce
248 pain significantly although it does reduce the severity of the responses during the first hour.
249 Only the combination of regional anaesthesia, 5 ml 2% lidocaine given s/c midway between
250 the horn base and the lateral eye canthus, with i.v. carprofen resulted in reduced struggling,
251 plasma cortisol and pain-related behaviours during the 24 hours after hot-iron disbudding,
252 and so can ensure good welfare in the calves.

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Fig. 1 - Degree of struggling (mean ±SD) during hot-iron disbudding (scale from 0 = no struggling to 5 = severe struggling). DA_2 (n=7) – disbudded with cornual nerve blocking with lidocaine; DAC_2 (n=7) – disbudded with cornual nerve blocking with lidocaine and carprofen; DC_2 (n=8) – disbudded with carprofen alone; D_2 (n=7) – disbudded with no treatment. ND_2 (n=8) – sham-disbudded.

Different letter indicates differences between groups.

Table 1 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in Experiment 1. DA_1: calves disbudded after treatment with lidocaine; DAC_1: calves disbudded after treatment with lidocaine and carprofen; D_1: calves disbudded without treatment; ND_1: calves sham-disbudded.

		Time from disbudding				
Group	n	-5 min	+ 1h	+ 3h	+ 6h	+ 24h
DA_1	6	18.21^{Aa} ± 5.53	16.95^{Aa} ± 5.53	25.17^{Aa} ± 5.53	28.19^{Aa} ± 5.53	17.11^{Aa} ± 5.53
DAC_1	6	24.00^{Aa} ± 5.12	15.54^{Aa} ± 5.12	21.11^{Aab} ± 5.12	14.87^{Aab} ± 5.12	35.98^{Bb} ± 5.12
D_1	7	15.64^{Aa} ± 5 .12	33.89^{Bb} ± 5.12	20.95^{Abab} ± 5.12	16.51^{Aab} ± 5.12	25.13^{Abab} ± 5.12
ND_1	8	10.64^{Aa} ± 5.12	7.17^{Aa} ± 5.12	10.09^{Ab} ± 5.12	12.40^{Ab} ± 5.12	15.66^{Aa} ± 5.12

Different lower case superscript letters indicate difference between groups for which $P<0.05$

Different upper case superscript letters indicate difference across time for which $P<0.05$.

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387 Table 2 – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head
388 rub and transitions from standing to lying) for calves disbudded with hot-iron in
389 Experiment 1. Observational period: 15 min. Treatment groups: DA_1 – calves disbudded
390 after treatment with lidocaine; DAC_1 – calves disbudded after treatment with lidocaine and
391 analgesia (carprofen); D_1 – calves disbudded without treatment; ND_1 – calves sham-
392 disbudded.

		Time from disbudding				
Group	n	+15m	+1 H	+ 3H	+ 6H	+ 24H
DA_1	6	1.50 ±0.33^{aA}	4.00 ±0.59^{aA}	4.00 ±0,42^{aA}	2.83 ±0.6^{aAB}	0.33 ±0.25^{aB}
DAC_1	7	0.57 ±0.53^{bA}	0.57 ±0.55^{bA}	0.57±0.37^{bcA}	1.00 ±1.29^{bA}	0.43 ±0.24^{aA}
D_1	7	6.14 ±1.35^{cA}	4.43 ±1.55^{aB}	1.43 ±0.98^{bC}	1.86 ±0.37^{abC}	0.43 ±0.24^{aC}
ND_1	8	0.57 ±0.53^{bA}	0.29 ±0.55^{bA}	0.14 ±0.38^{cA}	0.43 ±0.37^{bA}	0.43 ±0.24^{aA}

Different lower case superscript letters indicate difference between groups for which P<0.05

Different upper case superscript letters indicate difference across time for which P<0.05.

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404 Table 3 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in
405 Experiment 2. DA_2: disbudded after treatment with lidocaine; DAC_2: disbudded after
406 treatment with lidocaine and analgesic (carprofen); DC_2: disbudded after treatment with
407 analgesic (carprofen); D_2: calves disbudded without treatment; ND_2: calves sham-
408 disbudded.

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		Time from disbudding			
Group	n	-5 min	+ 10 min	+ 30 min	+ 50 min
DA_2	7	12.94 ±6.47^{aAC}	44.94 ±6.47^{aB}	15.16 ±6.47^{aA}	8.31 ±6.47^{aC}
DAC_2	7	19.06 ±6.47^{aAB}	32.69 ±6.47^{acA}	12.76 ±6.47^{aBC}	8.10 ±6.47^{aC}
DC_2	8	15.14 ±6.05^{aA}	83.77 ±6.05^{bB}	91.69 ±6.05^{bB}	72.02 ±6.47^{bB}

D_2	7	22.53	±6.47^{aA}	85.12 ±34.24^{bB}		122.18 ±6.47^{cC}	68.90 ±6.47^{bB}
ND_2	8	19.23	±6.05^{aA}	14.17 ±6.05^{cA}		14.49 ±6.05^{aA}	11.76 ±6.05^{aA}

Different lower case superscript letters indicate difference between groups for which P<0.05.

Different upper case superscript letters indicate difference across time for which P<0.05.

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421 Table 4 – Incidence (mean ±SD) of four different behaviours (head shake, ear flick, head
422 rub and transitions from standing to lying) for calves disbudded with hot-iron in
423 Experiment 2. Observational period: 10 min. Treatment groups: DA_2 – calves disbudded
424 after treatment with lidocaine; DAC_2 – calves disbudded after treatment with lidocaine and
425 analgesia (carprofen); $DC_{2:}$ disbudded after treatment with analgesic (carprofen); D_2 –
426 calves disbudded without treatment; ND_2 – calves sham-disbudded.

		Time from disbudding		
Group	n	0 - 10m	+20 - 30m	+40 - 50m
DA_2	7	1.71 ±1.11^{a}	0.71 ±0.76^{a}	2.14 ±1.35$^{bc\ ab}$

DAC_2	7	1.29	±1,50[a]	1.00 ±1.15[a]	0.86 ±1.21[cd b]
DC_2	8	4.00	±1.41[b]	2.88 ±0.99[b]	2.88 ±1.13[ab ac]
D_2	7	6.14	±2.12[b]	4.86 ±1.46[c]	5.00 ±2.83[a c]
ND_2	8	1.75	±1.04[a]	0.88 ±0.64[a]	0.63 ±0.74[d]

Different lower case superscript letters indicate difference between groups for which P<0.05. No differences along time within each group were found.

Table 5 – Mean ±SD plasma cortisol (nmol/L) of calves disbudded with hot-iron in Experiment 3. DA_3: disbudded after treatment with lidocaine; ND_3: calves sham-disbudded.

		Time from dehorning				
Group	n	-5 min	+ 90 min	+ 120 min	+ 150 min	Mean post-disbudding
DA_3	8	14.11 ±3.91[aA]	25.56 ±3.91[aBC]	18.50 ±3.91[aAB]	31.37 ±3.91[aC]	25.15 ±3.91[aB]
ND_3	5	14.24 ±4.52[aA]	15.35 ±4.52[aA]	17.84 ±4.52[aA]	17.71 ±4.52[bA]	16.97 ±4.52[bA]

Different lower case superscript letters indicate difference between groups for which P<0.05.

Different upper case superscript letters indicate difference across time for which P<0.05.

	-15m	90m	120m	150m
A	$14.11^{Aa} \pm 3.91$	$25.57^{BCa} \pm 3.91$	$18.50^{Aba} \pm 3.91$	$31.37^{Ca} \pm 3.91$
ND	$14.24^{Aa} \pm 4.52$	$15.09^{Aa} \pm 4.52$	$16.48^{Aa} \pm 4.52$	$17.26^{Ab} \pm 4.52$

Table 6 – Incidence (mean ±SD) of four different behaviours (head-shake, ear-flick, head-rub and transitions from standing to lying) for calves disbudded with hot-iron in Experiment 2. Observational period: 15 min. Treatment groups: DA_3 – calves disbudded after treatment with lidocaine; ND_3 – calves sham-disbudded.

		Time from dehorning		
Group	n	+90 m	+ 120 min	+ 150 min
DA_3	8	3.50 ± 3.21^{a}	3.00 ± 2.14^{a}	1.75 ± 1.49^{a}
ND_3	5	0.33 ± 0.58^{b}	0^{b}	0.67 ± 0.58^{a}

Different lower case superscript letters indicate difference between groups for which P<0.05

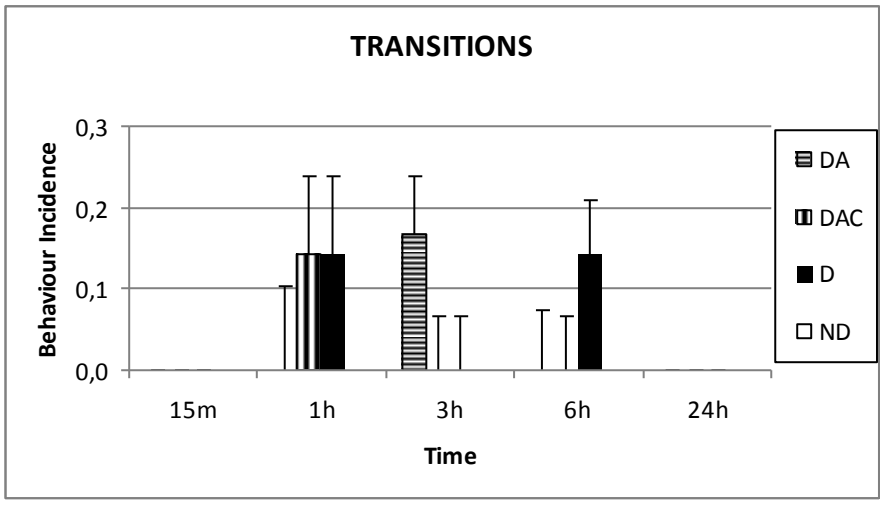

Fig. 1 – Incidence of transitions shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). For all comparisons p>0.05.

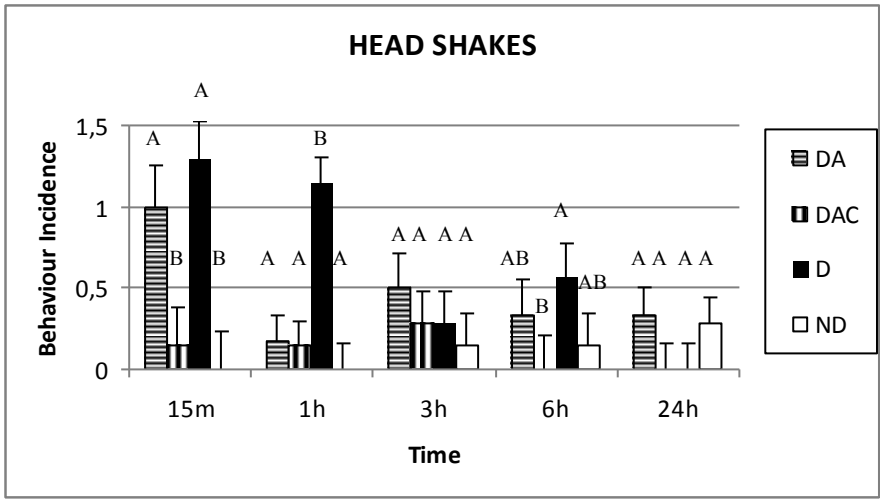

Fig. 2 – Incidence of head shakes shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences (p<0.05).

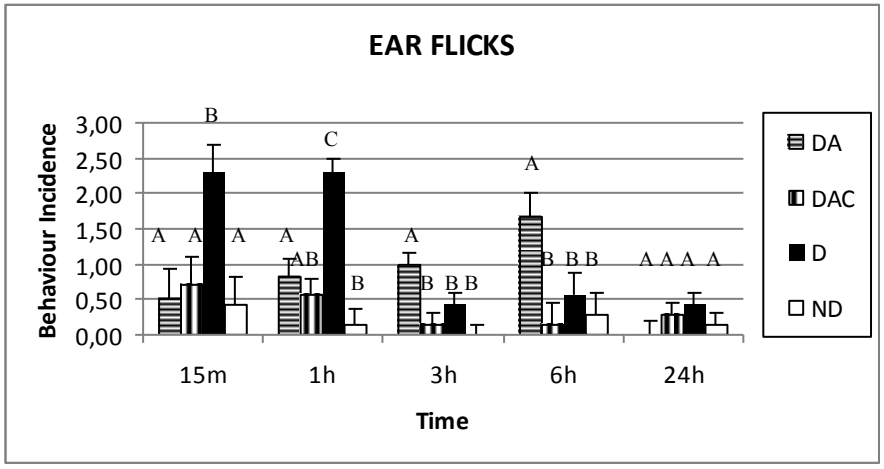

Fig. 3 – Incidence of ear flicks shown by calves' hot-iron disbudded after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences $p<0.05$.

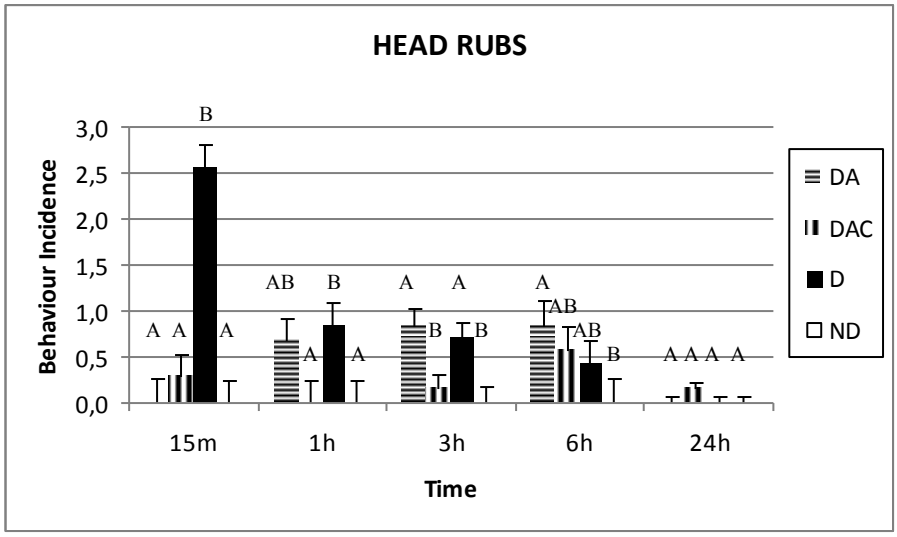

Fig. 4 – Incidence of head rubs shown by calves' hot-iron disbudded after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences $p<0.05$.

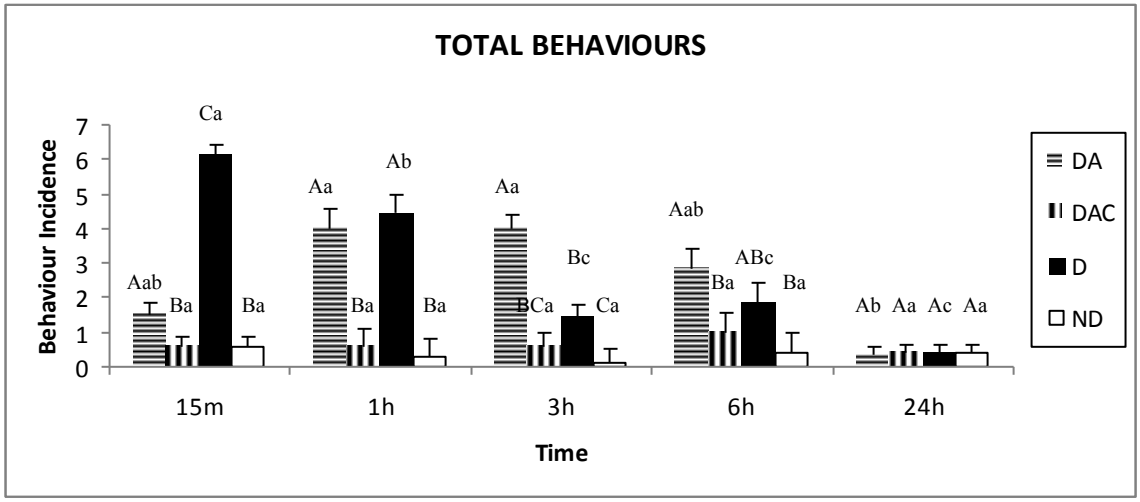

Fig. 5 – Total behaviours shown by hot-iron disbudded calves after local anaesthesia (DA), local anaesthesia plus carprofen (DAC), no treatment (D) or sham-disbudded (ND). Different upper case letters indicate differences at each time and different lower case letters indicate differences across time ($p<0.05$).