



Behaviour of tail-docked lambs tested in isolation

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Abstract

Abstract: The aims of the current study were to detect behavioural indicators of pain of tail-docked sheep tested in isolation and to determine the relationship between behaviour and the pain levels to which they were exposed. Twenty-four female lambs, randomly assigned to four pens, had their tail docked with a rubber ring (TD; n = 6) without pain control procedures, TD with anaesthesia (TDA; n = 6) or TD with anaesthesia and analgesia (TDAA; n = 6). Additionally, six lambs handled but without tail docking or application of pain relief measures were used as the control (C). On the day prior (Day -1) to the TD and on days 1, 3 and 5 post-procedure, each lamb was individually removed from its group and underwent a 2.5 min open field test in a separate pen. Frequencies of behaviours such as rest, running, standing, walking and exploring were directly observed. Frequencies of exploratory climbs (ECs) and abrupt climbs (ACs) over the testing pen's walls were video-recorded. Data were analysed using generalised linear mixed models with repeated measurements, including treatment and day as fixed effects and behaviour on Day -1 as a linear covariate. Control and TDAA lambs stood more frequently than TD lambs. TD lambs performed significantly more ACs compared to all other treatment groups. No other treatment effects were detected. A day effect was detected for all behaviours, while the EC frequency was highest for all tail-docked lambs on Day 5. Findings suggest that standing, ACs and ECs could be used as potential indicators of pain in isolated tail-docked lambs. However, differences in ECs between treatments only appeared 3 d after tail docking.

Keywords

behaviour • isolation • pain • sheep • tail docking

Introduction

The capacity to identify pain has important and practical implications for adequate pain management in farm practices to reduce suffering in animals (Morris *et al.*, 1994), as pain can be a major welfare concern in most production systems (EFSA, 2014). Farm animals, including sheep (*Ovis aries*), undergo potentially painful husbandry practices. However, determining the effects of potentially painful procedures on the welfare of farm animals is difficult.

Detection of pain signs in sheep is hindered not only by the animals' individual differences in reactions to threatening situations (Wemelsfelder and Farish, 2004), as well as differences in damaged tissues and ways in which the damage occurs (Kent *et al.*, 2000), but also by the effect of evolutionary pressures to hide pain to diminish predation risk (Butler and Finn, 2009). It has been speculated that showing an abnormal behaviour in response to pain may cause isolation from the group and increase predation risk (Dwyer, 2004). To reduce predation risk, an ability to temporarily displace awareness of pain can be developed (Rutherford,

2002). Once the animal is away from the danger, an elevated nociception takes place, as normal activity could aggravate the injury (Zimmerman, 1986).

A common procedure used in sheep farming is tail docking, which is considered a painful procedure (Sutherland and Tucker, 2011). Tail docking is performed to reduce fly strike risks and to improve fertility (French *et al.*, 1994; Webb-Ware *et al.*, 2000). If necessary, in these two cases, it is recommended to dock the tail of young animals up to 2 mo of age (Farm Animal Welfare Council [FAWC], 2009), as it is considered to be less painful (Molony *et al.*, 1993). Empirical data suggest however that younger lambs suffer from the same pain responses as older lambs (Dwyer, 2008) and are not less sensitive to acute pain (Guesgen *et al.*, 2011). Therefore, there is a need for appropriate husbandry recommendations for lambs of all ages.

Most previous studies have focused on observing tail-docked sheep while the procedure was performed, or after treatment application returning to their usual social environment (e.g. Graham *et al.*, 1997, 2002; Kent *et al.*, 1998; Grant, 2004;

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McCracken *et al.*, 2010). Under these conditions, tail-docked lambs have been widely described as showing agitation, as well as lateral and ventral recumbency, in addition to bleating, lip curling, kneeling, knee walking, writhing and other abnormal postures indicative of “intense pain” and “marked distress” within the first hours after conducting the procedure (i.e. Mellor and Murray, 1989; Molony *et al.*, 2002; Lomax *et al.*, 2010).

The effect of the pain of tail docking on lambs’ behavioural or postural abnormalities could be observed clearly within the first 3 h after treatment application, while afterwards, a decrease in acute pain is usually assumed (Graham *et al.*, 1997; Kent *et al.*, 1998; Molony *et al.*, 2002; Grant, 2004; McCracken *et al.*, 2010). Lambs may be immunologically stressed around the time of tail docking as a result of sub-clinical disease (Roger, 2008). However, ischaemic necrosis around the tail-docking area was still observed at 4 wk after treatment application, with frequent reddening, swelling, inflammation and minor infection of the skin immediately proximal to the ring, related to milder pain (Lomax *et al.*, 2010).

No information could be found regarding the behavioural responses of tail-docked lambs under the stress of isolation post-procedure and whether such behavioural response would depend on the varied experienced pain levels. Understanding such relationships was of particular interest and importance for pain evaluation under on-farm conditions, because most procedures are performed on individually handled sheep, separated from the flock.

With the twin objectives of detecting behavioural indicators of pain of isolated sheep and determining whether their behaviour depends on the pain levels, an experiment was conducted using tail docking in lambs. The responses of 1 mo-old lambs, tail docked using rubber rings, with or without the application of pain control measures, were studied for 5 d after treatment application. Anaesthesia and analgesia have been shown to decrease pain reactions in sheep, thus allowing the differentiation of applied pain levels (Sutherland and Tucker, 2011). We hypothesised that lambs that underwent tail docking with rubber rings under controlled pain levels will differ in their behavioural response to pain relative to sham tail-docked lambs, all tested under isolation.

Materials and methods

Animals and facilities

This study was carried out at the experimental dairy sheep farm of Neiker-Tecnalia (Arkaute, Spain) and complied with the requirements of the European Directive 86/609/ECC regarding the protection of animals used for experimental

and other scientific purposes. Thus, 24 Latxa female lambs (*Ovis aries*), born between 23rd and 30th January 2012, were used. No twin pairs of lambs were included in this study. Lambs were individually identified with numbered plastic ear tags. Then, 3 d prior to the start of the experiment, the lambs were removed from a single flock, in which they were kept after separation from the dam, and housed at a stocking density of 1 m² per lamb, in four pens (2 × 3 m) with six lambs each. The pens were located in a barn with solid walls and windows allowing natural lighting. The pens were constructed with 1.5 m high, grey synthetic plastic (polyvinyl chloride [PVC]) panels and were provided with straw bedding, a feeder and a drinker each. The straw was checked daily and fresh straw was added as required to maintain good bedding conditions. Feeding was based on ad libitum access to hay and concentrate. Prior to the start of the experiment, lambs were weighed (mean ± s.e.: 18.8 ± 0.9 kg). The treatments were applied when the animals were 47 ± 2 d old.

Experimental treatments

All 24 lambs were, following the power analysis, randomly divided in groups of six, balancing groups according to the lambs’ body weights. Three treatments were used for this study: tail docking with a rubber ring (TD; n = 6) without pain control procedures; TD with anaesthesia (TDA; n = 6); or TD with anaesthesia and analgesia (TDAA; n = 6). Additionally, six lambs handled but without tails docked or application of pain relief measures were used as control (C). Each group was allocated to one of four pens, to which one of the treatments was assigned.

The TD treatment was performed with an elastrator to stretch the elastic band around the tail. For the TDA and TDAA treatments, the drugs administered, their doses and administration sites are presented in Table 1. Bupivacaine hydrochloride, known to have rapid and long-lasting (up to more than 5 h) effects (Babst and Gilling, 1978), was used as a local anaesthetic agent, in combination with epinephrine (Inibsacain® 0.50% PLUS; Inibsa, Barcelona, Spain), to improve the action of epidural local anaesthetics, having its peak 5 min after administration (Ratajczak-Ensleme *et al.*, 2007). In the TDAA treatment, flunixin meglumine (Finadyne®; Intervet Shering Plough Animal Health, Madrid, Spain) was used in combination with bupivacaine hydrochloride and epinephrine (Inibsacain® 0.50% PLUS; Inibsa, Barcelona, Spain). Flunixin meglumine is a potent non-steroidal anti-inflammatory drug, whose analgesic efficacy has been confirmed in both laboratory animals and clinically in domestic species. This anaesthetic agent peaks 12–16 h post-administration, and the effects last for 24–36 h (Ciofalo *et al.*, 1977; Welsh and Nolan, 1995). All tail docking procedures were performed on Day 0, under a veterinarian’s supervision.

Table 1. Protocol of the treatments applied to 24 female lambs with or without their tails docked

Treatment	Code	Number of animals	Protocol	Remarks
Control	C	6	Catching the animal	-
Only tail docking	TD	6	Catching the animal and placing the rubber ring on the tail	-
Anaesthesia	TDA	6	Catching the animal and placing the rubber ring on the tail Bupivacaine hydrochloride (0.50%) and epinephrine bitartrate (1:200,000) 1.5 mL per animal via subcutaneous injection, precisely in the region proximal to the placement of the rubber ring	Treatment applied 2 min before placing the rubber ring on the tail
Anaesthesia + analgesia	TDAA	6	Catching the animal and placing the rubber ring on the tail Anaesthesia protocol was the same as for TDA group Additional application of 2.5 mL meglubine flunexin (intramuscular)	Treatment applied 2 min before placing the rubber ring on the tail

TD = tail docked; TDA = TD with anaesthesia; TDAA = TD with anaesthesia and analgesia.

Experimental procedures

In order to test indicators of pain in the lambs and to avoid pseudo-replication issues (Hurlbert, 1984), each lamb was treated and tested individually, without having visual contact with the group mates. Behavioural testing of all individuals was carried out on days -1, 0, 1, 3 and 5. All animals were removed from each home pen and moved to a nearby waiting pen (measuring 1 × 2 m). Immediately after arrival, one of the lambs was taken at random from the waiting area and placed, individually, in an adjacent open field testing arena (measuring 2 × 3 m). The remaining individuals were grouped and kept in the holding pen. The testing arena was built with solid PVC, had 1.5-m-high walls and was provided with straw litter. No water or feed was provided in the testing arena. Behavioural observations were performed by the same person on all the testing days and consisted of 2.5 min of continuous direct sampling observations. The behavioural ethogram included the following behaviours: run, stand, walk, explore, rest and urinate (Dwyer, 2004). Data were collected using the software Chickitizer (Sanchez and Estevez, 1998), which allows the collection of the behaviour and the location of the individual of interest in a Cartesian coordinate system (XY-coordinates). Spatial data will be reported in a separate manuscript. Behavioural changes observed in the current study were very frequent and had short durations; therefore, we considered them as events. The behavioural sequences were also video-recorded (Panasonic HDC-HS80, Osaka, Japan) as a precaution in case behavioural changes would occur too fast to be accurately recorded. Due to unexpected behaviours occurring during tests (i.e. exploratory climbs [ECs] and abrupt climbs (ACs) on the walls, defined as a climb on the pen walls requiring at least both front legs to be off the ground), and the fact that both types of climbs occurred very fast and frequently, the video recordings were used to assess their frequencies during the 2.5 min observations. Therefore, we distinguished these two types of climbs due to observed

differences in duration and their type, which are confirmed in the literature (Dodd *et al.*, 2012). ECs were defined as climbs that lasted longer than 2 s in which lambs were upright on their back legs, with the front legs placed on the pen walls. Abrupt climbs were defined as climbs that lasted up to 2 s consisting of a vigorous run ending with a jump on the pen wall, in which the individual attempted to hook with the legs to the top of the wall.

To minimise the stress caused by the novel testing environment, all lambs were exposed to a testing arena that was similar – in terms of length and conditions – to the test (2.5 min) in order to habituate them to the testing conditions. The habituation took place during two consecutive days before the onset of the study, and it was performed in the presence of the observer standing outside the testing arena. On each day of the experimental period, the treatment group testing order and the individuals within the group were selected at random. On Day 0, treatment application order and consecutive testing order were also random. The testing procedure was the same for all days of data collection, except for Day 0, for which prior to testing, each lamb underwent the treatment procedure. The testing took no more than 2 min per individual. After testing ended, the lamb was redirected to the holding pen, and a new individual was placed in the testing arena. The testing procedure was repeated until all lambs were tested.

Statistical analysis

From the continuous direct observations, the frequencies of the behaviours run, stand, walk, explore, rest and urinate, as well as frequencies of ECs and ACs, were recorded per lamb for each testing period. The frequencies for each particular behaviour and individual were not standardised according to the total frequency of behaviours per test because the duration of tests was equal for all lambs. A generalised linear mixed model analysis was performed,

including treatment, day and their interaction as fixed factors, and lamb, nested within treatment, as a random factor. The validity of the models was checked by using Akaike's information criterion. Values obtained on Day -1 were included as linear covariates into the models. PROC GLIMMIX of SAS v9.3 (SAS, 2003) (SAS Institute Inc., Cary, NC, USA) was used, with spatial power matrix accounting for uneven time distances between days, including day as repeated measure. The least square means for all significant effects in the models ($P < 0.05$) were computed using the LSMEANS option. The trend of a significant effect was considered for $P < 0.10$.

Results

The results of the statistical analysis are presented in Table 2. There was a treatment-by-day interaction for EC frequency (Table 2, Figure 1). Significant differences across treatments were only evident on Day 5, with C lambs showing significantly lower frequencies of ECs when compared to TD, TDA or TDAA groups. There were no differences in the frequency of ECs across days for the C lambs, whereas a clear increment in this frequency was observed on Day 5 for all other treatments, especially for TD (Figure 1).

Table 2. Results of analysis of variance for the frequency of behaviours, such as explore, run, stand and walk, as well as the frequency of exploratory and abrupt climbs, performed by 24 female lambs with or without their tails docked¹ measured for the five experimental days

Behaviour ²	Analysis of variance components					
	Treatment		Day		Treatment x day	
	F-value	P	F-value	P	F-value	P
Explore	$F_{(3,19)} = 0.02$	0.997	$F_{(3,60)} = 5.14$	0.003	$F_{(9,60)} = 1.13$	0.356
Run	$F_{(3,19)} = 0.73$	0.554	$F_{(3,60)} = 9.21$	<.0001	$F_{(9,60)} = 0.64$	0.759
Stand	$F_{(3,19)} = 5.98$	0.005	$F_{(3,60)} = 3.36$	0.024	$F_{(9,60)} = 0.48$	0.880
Walk	$F_{(3,19)} = 1.14$	0.359	$F_{(3,60)} = 13.87$	<.0001	$F_{(9,60)} = 1.32$	0.2483
Exploratory climbs ³	$F_{(3,19)} = 0.94$	0.440	$F_{(3,60)} = 9.66$	<.0001	$F_{(9,60)} = 2.10$	0.043
Abrupt climbs ⁴	$F_{(3,19)} = 3.37$	0.040	$F_{(3,60)} = 0.63$	0.599	$F_{(9,60)} = 0.62$	0.775

¹Female lambs had their tails docked (TD) with a rubber ring (n = 6) without pain control procedures, TD with anaesthesia (n = 6) or TD with anaesthesia and analgesia (n = 6). Additionally, six lambs handled but without TD or pain reliever application were used as control (C).

²Each lamb was individually observed during a 2.5 min open field test.

³Exploratory climbs = climbs that lasted longer than 2 s in which lambs were upright on their back legs, with the front legs placed on the pen walls and their head over the fence.

⁴Abrupt climbs = climbs that lasted up to 2 s consisting of a vigorous run ending with a jump on the pen wall, in which the individual attempted to hook with the legs to the top of the wall.

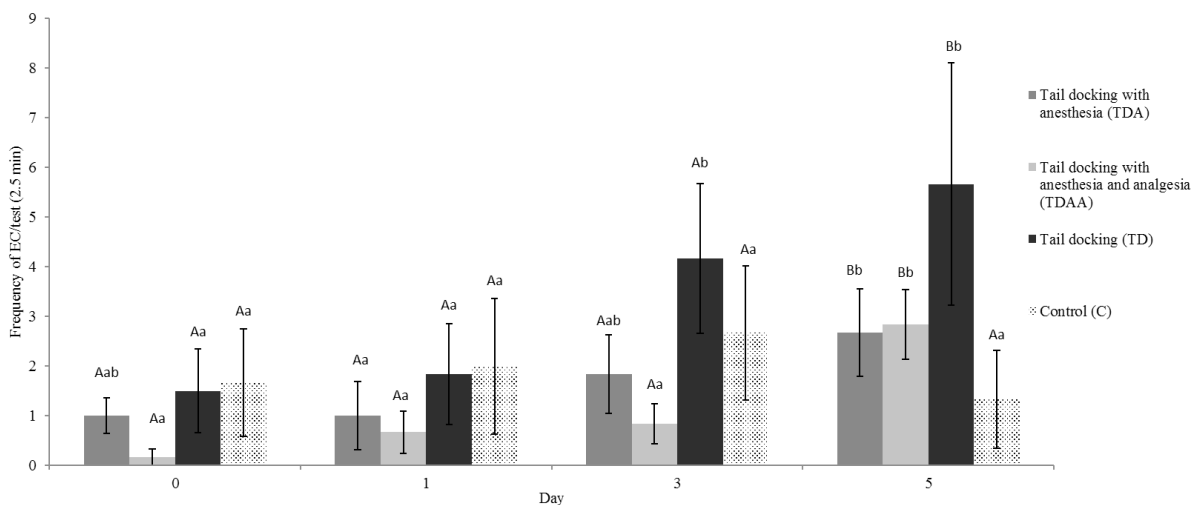


Figure 1. Treatment-by-day interaction on the mean frequency \pm s.e. of ECs per 2.5 min test. Major letters indicate significant LSM differences across treatments ($P < 0.05$) on a particular day. Within each variable, small letters indicate significant LSM differences across days ($P < 0.05$) for each treatment. EC = exploratory climbs; LSM = least squares mean.

There was an effect of tail-docking treatment on the frequency of standing (Figure 2), whereby C and TDAA lambs stood more frequently than TD lambs. In addition, TD lambs had a higher frequency of ACs, as compared to all other treatments (Figure 2). No other significant effects of treatment were detected. Resting and urination frequencies were close to zero and therefore disregarded.

An effect of day was detected for all indicators (exploring, running, standing, walking and ECs), except for ACs (Table 2). Independently of the treatment, lambs walked, ran and stood more, and explored less on Day 0 as compared to any other day (Table 3). We observed less ECs on the walls on the first 2 d (Day 0 and Day 1), than on days 3 and 5.

Discussion

This study aimed to detect behavioural indicators of pain in sheep that underwent tail docking while separated from their pen mates and to determine whether the lambs' behaviour depended on the varied experienced pain levels. This is relevant now due to the current attention of governmental agencies and civil society organisations to the improvement of farm animals' welfare by fulfilling set standards for rearing animals free from pain (FAWC, 2009). Our study was conducted on 24 Latxa female lambs tested prior and after undergoing tail docking, with or without pain control measures. We expected to find

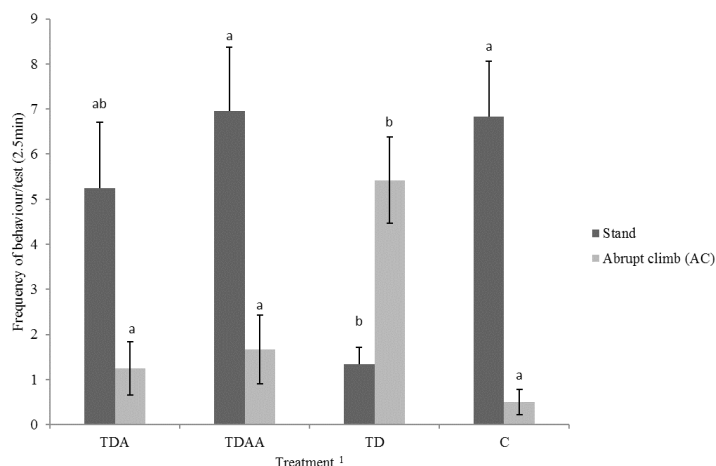


Figure 2. Effect of day on the mean frequency ± s.e. of standing and ACs per 2.5 min test. Within each variable, different letters indicate significant LSM differences across treatments ($P < 0.05$).

¹Female lambs had their tail docked (TD) with a rubber ring ($n = 6$) without pain control procedures, TD with anaesthesia (TDA; $n = 6$) or TD with anaesthesia and analgesia (TDAA; $n = 6$). Additionally, six lambs handled but without TD or pain reliever application were used as control (C). LSM = least squares mean.

Table 3. Frequency (least squares mean ± s.e.) of behaviours such as explore, run, stand and walk, as well as the frequency of exploratory and abrupt climbs, performed by 24 female lambs with or without their tail docked¹ measured for the five experimental days.

Behaviour ²	Day 0	Day 1	Day 3	Day 5
Explore	3.67 ± 0.81 ^a	8.71 ± 1.23 ^b	7.38 ± 1.14 ^{bc}	5.75 ± 1.01 ^c
Run	11.88 ± 2.12 ^a	4.46 ± 1.09 ^b	4.54 ± 1.02 ^b	3.71 ± 1.16 ^b
Stand	7.17 ± 1.24 ^a	4.33 ± 1.37 ^b	3.96 ± 1.10 ^b	4.92 ± 1.32 ^b
Walk	13.42 ± 1.78 ^a	4.13 ± 0.80 ^b	7.17 ± 1.08 ^c	7.83 ± 0.93 ^c
Exploratory climbs ³	1.08 ± 0.36 ^a	1.38 ± 0.45 ^a	2.38 ± 0.58 ^b	3.13 ± 0.75 ^b
Abrupt climbs ⁴	2.88 ± 0.89	2.46 ± 0.83	1.88 ± 0.83	1.63 ± 0.58

¹Female lambs had their tail docked (TD) with a rubber ring ($n = 6$) without pain control procedures, TD with anaesthesia ($n = 6$) or TD with anaesthesia and analgesia ($n = 6$). Additionally, six lambs handled but without TD or pain reliever application were used as control (C).

²Each lamb was individually observed during a 2.5 min open field test.

³Exploratory climbs = climbs that lasted longer than 2 s in which lambs were upright on their back legs, with the front legs placed on the pen walls and their head over the fence.

⁴Abrupt climbs = climbs that lasted up to 2 s consisting of a vigorous run ending with a jump on the pen wall, in which the individual attempted to hook with the legs to the top of the wall.

^{a-c}Means within a row with different superscripts differ ($P < 0.05$). Data were averaged across treatments for each time point.

clear behavioural differences across tail-docking treatments, soon after the procedure was applied, when the differences in pain levels were expected to be the highest.

None of the studied behavioural indicators, except for ECs, was affected by the treatment-by-day interaction. It is possible that in our study, isolated lambs might have experienced a stress-induced analgesia-like syndrome, as social isolation is considered very distressing for sheep (Boissy *et al.*, 2005). It may be possible that the combination of social isolation stress and pain originating from the procedure was sufficient to induce some form of natural analgesia during the first 3 d of testing, although not sufficient to totally overcome the reactions detected in tail-docked lambs. When the acute pain transmutes into chronic pain, behavioural reactions may increase (Rivat *et al.*, 2007). A similar process might have occurred in the TD lambs in our study, as probably the presence of ring could still be perceived, but pain was no longer acute due to the appearance of ischemic necrosis (Lomax *et al.*, 2010). It may be further speculated that tail-docked lambs showed lower ability or required a longer time to habituate to the testing conditions as compared to control lambs because the frequency of ECs in control lambs remained on a similar level across days of observations, while it increased for treated lambs. It may be possible that isolation will always remain a strong testing condition to which lambs will have difficulties to habituate. As the tests were subsequently repeated, a clear reduction in the behavioural activity was observed during the experiment, suggesting that lambs may have become habituated to the isolation testing conditions, and an extinction mechanism of the response may have occurred (Erhard *et al.*, 2006). Even though the habituation period prior to testing was performed according to previously accepted methods (Forkman *et al.*, 2007), all lambs showed decreased activity levels over subsequent testing days independent of the treatment. This may indicate that the habituation was not completed before the testing started.

We were not able to clearly determine whether ECs were attempts to establish social contact with flock mates, or whether they were related to exploration of the environment. However, distinguishing vertical from horizontal movements of isolated individuals has been previously reported to provide important information regarding their state (Canini *et al.*, 2009). Therefore, further attention should be paid to EC frequency as it was the only indicator that we found to be affected by the treatments over time.

Although no treatment effects were detected for the frequency of walking or running, differences in ACs and standing were observed between control and lambs tail docked without any pain relief measures. The lower frequency of standing, as well as high AC frequency, may be indicative of restlessness, related to escape attempts. It may also be a part of a natural fight-or-flight reaction, as the result of the combined effects

of pain caused by tail docking without pain relief treatment and of social isolation. These results agree with previous studies in which high locomotor activity was considered a sign of distress in the context of social isolation (Boissy *et al.*, 2005), whereas increased incidence of active behaviours was considered an attempt to escape or remove the perceived pain (Graham *et al.*, 1997).

High frequencies of running and walking, as well as a low frequency of exploration, were generally observed on the day of treatment application in all groups regardless of the treatment. Initial high activity levels observed in this study would suggest that lambs were reacting more to the distress caused by isolation, than to the potential pain caused by the treatments. González *et al.* (2013) reported similar results whereby stress reactions due to social isolation were substantially stronger than those elicited by the presence of the sudden stimulus.

Interestingly, under the isolation conditions in which the study was performed, we did not observe any of the indicators of pain, such as rolling, foot stamping, kicking and easing quarters, commonly reported for group-housed, tail-docked lambs (Molony and Kent, 1997; Kent *et al.*, 1998). The only exception was restlessness on any of the days, including Day 0. This would suggest that lambs simultaneously subjected to stress and pain conditions may have diverse coping mechanisms, depending on their social environment.

The results we obtained are relevant to the ongoing discussions regarding pain assessment in sheep and provide new perspectives on the impact that environment may have on the reliability of interpretation of the pain reactions. In practice, when evaluating the condition of animals under suspected pain – usually separated from the flock – for veterinary examination, decrease in pain reactions should be considered, as observed in the current study. On the other hand, it may be possible that if animals would be more used to stockpersons, then this mechanism could be bypassed, and the real health status of the animal can be correctly perceived. The delayed and elevated nociception should also be accounted for when scoring pain in animals, because what is considered as an extreme pain response can be a delayed effect of earlier acute pain. Finally, the reason for the increase in EC frequency on Day 5 is unclear. For the moment, we question whether the observed reactions are related to a potential transition from acute to chronic or no pain, or should they rather be explained by habituation to the testing procedure.

Conclusions

The hypothesis about differences in behaviour and activity levels between tail-docked lambs in the presence or absence of pain control measures and control lambs was partially

confirmed in the current study, because there were differences in terms of ACs and standing frequency between TD and C lambs. ACs might equally represent strategies implemented to avoid isolation (i.e. linked to isolation stress), as to express pain. Differences in the frequency of ECs between treatments, being highest for tail-docked lambs, were only found on Day 5. This might suggest the effect of isolation on pain perception up to 5 d after treatment application. Testing lambs under isolation has a marked effect on their pain responses and prevents the detection of the indicators of pain from their behaviour. These findings might have important implications for the design of further pain-exploring studies, as well as for everyday on-farm practices.

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