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**Mechanical/thermal sensitivity and superficial temperature in the stump of long-term tail-docked dairy cows**

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**Background.** Tail docking of dairy cows is a painful procedure that affects animal welfare. The aims of this study were first to evaluate the response to mechanical and thermal stimulation, and second to determine the superficial temperature of the stump of tail-docked dairy cows. **Methods.** One hundred and sixty four dairy cows were enrolled. From these, 133 cows were assigned to the tail-docked group (TD) and 31 cows were selected as control animals (C). The following sensory assessments to evaluate pain in tail-docked cows were performed. Sensitivity of the tail region in both groups of animals was evaluated using a portable algometer. Cold and heat sensitivity assessment was performed using a frozen pack (0ºC) and warm water (45ºC), respectively. Pinprick sensitivity was evaluated using a Wartenberg neurological pinwheel. Superficial temperature was evaluated using a thermographic camera. All sensory assessments and superficial temperature were evaluated in the ventral surface of the tail stump (TD) and tail (C). **Results.** Analysis revealed a significant effect of condition on the pressure pain threshold with significantly lower values necessary to obtain a withdrawal response in TD cows (5.97±0.19 kg) compared to C cows (11.75±0.43 kg). Chi Square test revealed that there was a significant association between heat sensitivity and condition with 29.3% of TD cows responding positively. Nonetheless, no association was found between condition and cold sensitivity. Similarly, after pinprick sensitivity test was performed, 93.2% of TD cows elicited a positive response to stimulation. This sensory testing was significantly associated with the condition. There was a significant effect of condition on superficial temperature, where TD cows had significantly lower temperature (26.43±0.27 ºC) when compared to C animals (29.86±0.62 ºC). **Discussion.** Pressure pain threshold values in both groups of animals were higher than those previously reported for tail docked pigs, sows and cows. In contrast, pinprick stimulation evaluates the presence of punctate mechanical hyperalgesia/allodynia, usually related to traumatic nerve injury, and this...
association may reveal that it is possible that these animals developed a disorder associated to the development of a tail stump neuroma and concurrent neuropathic pain, previously reported in tail-docked lambs, pigs and dogs. Thermal sensitivity showed that tail-docked cows responded positively only to heat stimulation. These findings suggest that long-term tail-docked cows could be suffering hyperalgesia/allodynia, which may be indicative of chronic pain. Lower superficial temperature in the stump may be associated to sympathetic fiber sprouting in the distal stump, which can lead to vasoconstriction and lower surface temperatures. Further studies are needed in order to confirm neuroma development and adrenergic sprouting.
MECHANICAL/THERMAL SENSITIVITY AND SUPERFICIAL TEMPERATURE IN
THE STUMP OF LONG-TERM TAIL-DOCKED DAIRY COWS

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Abstract

**Background.** Tail docking of dairy cows is a painful procedure that affects animal welfare. The aims of this study were first to evaluate the response to mechanical and thermal stimulation, and second to determine the superficial temperature of the stump of tail-docked dairy cows.

**Methods.** One hundred and sixty four dairy cows were enrolled. From these, 133 cows were assigned to the tail-docked group (TD) and 31 cows were selected as control animals (C). The following sensory assessments to evaluate pain in tail-docked cows were performed. Sensitivity of the tail region in both groups of animals was evaluated using a portable algometer. Cold and heat sensitivity assessment was performed using a frozen pack (0ºC) and warm water (45ºC), respectively. Pinprick sensitivity was evaluated using a Wartenberg neurological pinwheel. Superficial temperature was evaluated using a thermographic camera. All sensory assessments and superficial temperature were evaluated in the ventral surface of the tail stump (TD) and tail (C).

**Results.** Analysis revealed a significant effect of condition on the pressure pain threshold with significantly lower values necessary to obtain a withdrawal response in TD cows (5.97±0.19 kg) compared to C cows (11.75±0.43 kg). Chi Square test revealed that there was a significant association between heat sensitivity and condition with 29.3% of TD cows responding positively. Nonetheless, no association was found between condition and cold sensitivity. Similarly, after pinprick sensitivity test was performed, 93.2% of TD cows elicited a positive response to stimulation. This sensory testing was significantly associated with the condition. There was a significant effect of condition on superficial temperature, where TD cows had significantly lower temperature (26.43±0.27 ºC) when compared to C animals (29.86±0.62 ºC).

**Discussion.** Pressure pain threshold values in both groups of animals were higher that those previously reported for tail docked pigs, sows and cows. In contrast, pinprick stimulation evaluates the presence of punctate mechanical hyperalgesia/allodynia, usually related to traumatic
nerve injury, and this association may reveal that it is possible that these animals developed a
disorder associated to the development of a tail stump neuroma and concurrent neuropathic pain,
previously reported in tail-docked lambs, pigs and dogs. Thermal sensitivity showed that tail-
docked cows responded positively only to heat stimulation. These findings suggest that long-term
tail-docked cows could be suffering hyperalgesia/allodynia, which may be indicative of chronic
pain. Lower superficial temperature in the stump may be associated to sympathetic fiber
sprouting in the distal stump, which can lead to vasoconstriction and lower surface temperatures.
Further studies are needed in order to confirm neuroma development and adrenergic sprouting.

Key Words: dairy cow, pain, tail-docked

Introduction
Tail docking of dairy cows was first introduced in New Zealand in the early 1900s, and comprises
the removal of an important part of the tail in order to improve udder and milk hygiene, and
promoting personnel comfort during the milking process (Schreiner & Ruegg, 2002a; Sutherland
& Tucker, 2011; Aubry, 2005). It has also been associated with cleaner cows by reducing the
exposure to manure and mud (Stull et al., 2002). Nonetheless, different studies have not found
differences in animal cleanliness, milk quality and somatic cell count between docked and non-
docked animals (Tucker, Fraser & Weary, 2001; Schreiner & Ruegg, 2002a). Tail docking is
prohibited in some countries, including Denmark, Germany, Sweden, Scotland, and the United
Kingdom (Hepple & Clark, 2011). In Chile, a marked decrease in its practice has been observed,
nonetheless it is not currently forbidden. Chilean legislation indicates that painful procedures,
including ear tagging, tail docking, castration, and disbudding should be performed minimizing
pain and suffering (Chile, 2013).
Tail docking negatively impacts animal welfare (Stull et al., 2002). It has been reported that docked cows have increased fly loads leading to alterations of eating patterns with the consequently decrease in milk production and increased fly avoidance behavior (Phipps, Matthews & Verkerk, 1995; Eicher et al., 2001). Contradictory evidence suggests that tail docking induces pain with behavioral changes associated to the procedure. Docking has been related with the presence of restless behavior, dorsal and lateral tail movements (Eicher & Dailey, 2002; Tom et al., 2002; Eicher et al., 2006). According to Kroll et al. (2014) there is an increase risk for potential chronic pain development at the amputation site, which has not been evaluated thoroughly in cows from commercial dairy farms. The aims of this study were first to evaluate the response to mechanical and thermal stimulation, and second to determine the superficial temperature of the stump of tail-docked dairy cows.

**Materials & Methods**

**Animals and housing**

This study was conducted between November and December 2015 in a commercial farm with confined system located in Los Rios Region, southern Chile. The study was approved by the Ethics and Bioethics Committee of Animal Research of the Universidad Austral de Chile (MV.21.2015). A total of 164 Holstein dairy cows with a mean age of 6.21±1.91 years and a mean body weight of 423±26 kg were enrolled. Only cows without clinical signs of systemic disease during the last 15 days were selected. Cows were housed in a tie-stall, fed a total mixed ration (TMR) and milked three times a day. From these, 133 cows were assigned to the tail-docked group (TD) and 31 cows were selected as control animals (C) and identified using the ear tag farm number. Individual register showed that cows in the TD group were tail-docked at 12 month of age using a rubber band.
**Study design**

A clinical sensory assessment protocol was developed in order to evaluate the presence of pain in tail-docked cows. Prior to sensory testing, cows were habituated to the presence of the evaluator and experimental testing was performed during three consecutive days. After the morning milking, cows were allowed to return to their tie-stall individual cubicles and were restrained using a headlock self-locking system for sensory assessment. The same evaluator (RT) performed all the sensory assessments with the assistance of another researcher in charge of identify and register positive reactions to the sensory stimuli. In order to avoid stress in the animals, both researchers approached the animals in a calm and quiet manner.

**Sensory assessments**

None of the animals received analgesic treatment previous to the sensory evaluation. The following tests were performed:

**Pressure pain sensitivity**: Sensitivity of the tail region in both groups of animals was evaluated using a portable algometer (Wagner FDX 25 Compact Digital Force Gauge, Wagner Instruments, Riverside, CT, USA) with a 1 cm\(^2\) rubber probe. For each evaluation, the probe was constantly applied in the same topographical location and placed perpendicular to the skin. The amount of pressure applied during each evaluation was constantly increased at 500 grams of force per second in the ventral surface of the tail stump (TD) and tail (C), respectively, until a positive response was obtained. Each area was assessed five times at 60-second intervals. Lateral and ventral movement and/or withdrawal of the tail were considered positive responses, in which the pressure elicited by the algometer was immediately discontinued and pressure registered. The mean of 5 measurements per site was considered as a single value per tested cow.

**Thermal sensitivity**: Cold and heat sensitivity assessment was performed using a frozen pack (0\(^\circ\)C) and warm water (45\(^\circ\)C), respectively. Both stimuli were applied for 5 seconds in the ventral surface of the tail stump (TD) and tail (C), respectively, or until a positive response was
obtained. Lateral and ventral movement and/or withdrawal of the tail were considered positive responses.  

Pinprick sensitivity: The pinprick sensitivity was evaluated using a Wartenberg neurological pinwheel applied in the ventral surface of the tail stump (TD) and tail (C), respectively. Lateral and ventral movement and/or withdrawal of the tail were considered positive responses.  

Superficial temperature  
Superficial temperature was evaluated using a thermographic camera (FLIR® i5, Wilsonville OR, USA) calibrated with an emissivity (ε) of 0.95 according to the manufacturer. Images from the ventral surface of the tail were obtained at a distance of 10 cm. All the images were obtained before sensory stimulus were applied. Thermogram analysis was performed using the FLIR® Tools 5.4 software, and atmospheric temperature and relative humidity were included in the analysis. To come to a single representative value, the mean temperature obtained from 5 longitudinal lines along the ventral surface of the tail, was considered.  

Statistical analysis  
For each continuous variable, probability plots were generated to verify that data followed a normal distribution. Pressure pain threshold and superficial temperature were analyzed using analysis of covariance. The linear model included condition as fixed effect and age as covariate. For pinprick and thermal sensitivity, Chi Square tests were conducted to analyze for possible association between condition and sensitive stimulation. For all statistical procedures, the overall alpha was set to 0.05. The statistical analysis was performed using R Statistical Software (R Core Team, Vienna, Austria).  

Results
Analysis revealed a significant effect of condition (TD versus C) on the pressure pain threshold (P<0.001, $\eta_p^2=0.46$) with significantly lower values necessary to obtain a withdrawal response in TD cows (5.97±0.19 kg) compared to C cows (11.75±0.43 kg) (Fig. 1). Chi Square test revealed that there was a significant association between heat sensitivity and condition ($\chi^2 =10.36$, df=1, P=0.0013) with 29.3% of TD cows responding positively (Table 1). Nonetheless, no association was found between condition and cold sensitivity ($\chi^2 =3.46$, df=1, P=0.0629) (Table 1). Similarly, after pinprick sensitivity test was performed, 93.2% of TD cows elicited a positive response to stimulation. This sensory testing was significantly associated with the condition ($\chi^2 =7.87$, df=1, P=0.005). There was a significant effect of condition on superficial temperature (P<0.00000283, $\eta_p^2=0.13$), where TD cows had significantly (P<0.001) lower temperature (26.43±0.27 °C) when compared to C animals (29.86±0.62 °C) (Fig. 2).

Discussion

Painful procedures are performed in the dairy industry and they are often associated with the development of fear, distress and chronic pain of animals (Grandin 2015). Tail docking is a painful procedure that induces both acute and chronic pain, and leads to behavioral modifications and discomfort (Tucker, Fraser & Weary 2001). Different studies have confirmed the presence of acute pain and augmented animal activity, characterized by a marked increase in foot stomps following tail docking (Eicher & Dailey 2002; Schreiner & Ruegg 2002b; Tom et al. 2002). Nonetheless, sensory stimulation in long-term tail docked cows has rarely been performed. The aims of this study were first to evaluate the response to mechanical and thermal stimulation, and second to determine the superficial temperature of the stump of tail-docked dairy cows. Tail-docked cows showed significantly less pressure pain threshold compared to controls. This results are similar to those reported in pigs, in which mechanical sensitization of the tail stump
lasted for up to 16 weeks (Di Giminiani et al. 2017). Pressure pain threshold values in both
groups of animals were higher than those previously reported for tail docked pigs (Di Giminiani et
al. 2016), sows (Nalon et al. 2016) and cows stimulated using an algometer with a metal probe in
the third metatarsal bone (Raundal et al. 2014). The higher overall pressure values described in
this study could be related to the use of a rubber probe. According to Di Giminiani et al. (2016)
the use of different probes could be associated to an increase degree of response variability.
Similarly, Taylor and Dixon (2012) mention that an increase in probe diameter results in higher
variability. Another factors that may influence the higher values of pressure threshold presented
here may include skin thickness (Di Giminiani et al. 2016), individual variation (Nalon et al.
2016) and stress-induced hypoalgesia (Herskin, Munksgaard & Ladewig 2004). The significant
association between condition and heat sensitivity is similar to that reported by Eicher et al.
(2006) in which tail docked cows manifested lesser number of foot stomps, foot shifts and tail
swings. Nonetheless, in this study we did not find a significant association (P = 0.0629) between
condition and cold sensitivity. Moreover, here we report a significant association between
condition and pinprick sensitivity. Impaired sensitivity to pinprick has been previously reported
in amputated human patients (Kosasih & Silver-Thorn 1998). Pinprick stimulation evaluates the
presence of punctate mechanical hyperalgesia/allodynia, usually related to traumatic nerve injury
(Jensen & Finnerup 2014). This association may reveal that it is possible that these animals
developed a disorder associated to the development of a tail stump neuroma. Petrie et al. (1996)
indicate that tail docking would induce tissue damage that leads to neuromata development and
concurrent neuropathic pain. Moreover, neuroma development has been previously reported in
tail-docked lambs (French & Morgan 1992; Fisher & Gregory 2007), pigs (Herskin, Thodberg &
Jensen 2015; Kells et al. 2017) and dogs (Gross & Carr 1990). Peripheral neuromas occur in 10-
25% of human amputees, and are generally formed after injury or surgical procedures, resulting
in neuropathic pain, residual limb pain, functional impairment and psychological distress (Rajput,
Reddy & Shankar 2012), increasing sensitivity to mechanical and thermal stimulation (Toia et al. 2015; O’Reilly et al. 2016; Yao et al. 2017). Histopathological analysis confirmed the presence of neuroma in the tail stump of docked pigs one month after tail docking, characterized by marked nerve sheath and axonal proliferation (Sandercock et al. 2016). Moreover, another study in pigs identified age at time of the procedure as a factor that may influence the development of neuropathic pain (Di Giminiani et al. 2017). Nonetheless, cows in the present study were, on average tail docked 48 months before sensory evaluation. According to this, we believe that pain experienced by docked cows is similar to human phantom limb pain, which has also been associated with neuroma formation (Nikolajsen 2012). Phantom limb pain has been vastly studied in humans (Schley et al. 2008; Andoh et al. 2017; Yin et al. 2017). In cases of phantom limb pain, characteristic chronic neuropathic pain occurs in the amputation stump; and although this pain may decrease or eventually disappear over time, if continues for more than 6 month, the prognosis for pain decrease is poor (Kuffler 2017).

Surface temperature was significantly lower in the TD group compared to controls. Similar results were reported by Eicher et al. (2006), where the stump of docked heifers had approximately 2°C less than the underside of the tails of intact heifers. Similar results have been described in amputated humans, in which the stump of amputated limbs reflects lower superficial temperatures than the contralateral side using a temperature probe (Hunter, Kats & Davis 2005) and thermographic analysis (Harden et al. 2008). This decrease in temperature may be associated to sympathetic fiber sprouting in the distal stump, which can lead to vasoconstriction and lower surface temperatures (Harden et al. 2004). Similarly, Nascimento et al. (2015), after traumatic nerve injury confirms the presence of sympathetic sprouting in the skin that contributes to pain.

Conclusions

Results of the present study indicate that tail-docked cows had an increase response to mechanical stimulation characterized by lower pain pressure thresholds and a positive association
to pinprick sensitivity. Thermal sensitivity showed that tail-docked cows responded positively only to heat stimulation. These findings suggest that long-term tail-docked cows could be suffering hyperalgesia/allodynia, which may be indicative of chronic pain. Lower superficial temperature in the stump, could be associated to adrenergic tissue sprouting inducing peripheral vasoconstriction. Further studies are needed in order to confirm neuroma development and adrenergic sprouting.

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Least square means and standard error for pressure pain threshold in tail docked (n=133) and control cows (n=31). *Statistically significant differences between groups (P<0.001).
Figure 2

Least square means and standard error for superficial temperature in tail docked (n=133) and control cows (n=31). *Statistically significant differences between groups (P<0.001).
**Table 1**

Frequencies and percentages of sensory assessment in tail docked (n=133) and control cows (n=31).
Table 1. Frequencies and percentages of sensory assessment in tail docked (n=133) and control cows (n=31).

<table>
<thead>
<tr>
<th></th>
<th>Tail docked</th>
<th>Control</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive N (%)</td>
<td>Negative N (%)</td>
<td>Positive N (%)</td>
</tr>
<tr>
<td>Heat sensitivity</td>
<td>39 (29.3)</td>
<td>94 (70.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Cold sensitivity</td>
<td>31 (23.3)</td>
<td>102 (76.6)</td>
<td>2 (6.6)</td>
</tr>
<tr>
<td>Pinprick stimulus</td>
<td>124 (93.2)</td>
<td>9 (6.8)</td>
<td>23 (74.2)</td>
</tr>
</tbody>
</table>

*P values for Chi square test