

1 **MECHANICAL / THERMAL SENSITIVITY AND SUPERFICIAL TEMPERATURE IN**
2 **THE STUMP OF LONG-TERM TAIL-DOCKED DAIRY COWS**

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26 **Abstract**

27 **Background.** Tail docking of dairy cows is a painful procedure that affects animal welfare level.

28 The aims of this study were first to evaluate the response to mechanical and thermal stimulation,
29 and second to determine the superficial temperature of the stump of tail-docked dairy cows.

30 **Methods.** One hundred and sixty-four dairy cows were enrolled. From these, 133 cows were
31 assigned to the tail-docked (TD) group ~~(FD)~~ and 31 cows were selected as control animals ~~(C)~~.

32 The following sensory assessments to evaluate pain in tail-docked cows were performed.
33 Sensitivity of the tail region in both groups of animals was evaluated using a portable algometer.
34 Cold and heat sensitivity assessment was performed using a frozen pack (0°C) and warm water
35 (45°C), respectively. Pinprick sensitivity was evaluated using a Wartenberg neurological pinwheel.

36 Superficial temperature was evaluated using a thermographic camera. All sensory assessments and
37 superficial temperature were evaluated in the ventral surface of the tail stump (TD) and tail (C).

38 **Results.** ~~Analysis revealed a significant effect of condition on the P~~pressure pain threshold ~~with~~
39 ~~was significantly~~ lower values necessary to obtain a withdrawal response in TD cows (5.97 ± 0.19
40 kg) compared to ~~C~~control cows (11.75 ± 0.43 kg). ~~Chi-Square test revealed that there was a~~
41 ~~significant association between H~~heat sensitivity ~~and was higher in the condition~~TD cows
42 ~~compared to control cows~~ with 29.3% of TD cows responding positively. Nonetheless, no
43 association was ~~found present~~ between ~~condition~~tail docking and cold sensitivity. Similarly, after
44 pinprick sensitivity test was performed, 93.2% of TD cows elicited a positive response to
45 stimulation. ~~This sensory testing was significantly associated with the condition. There was a~~
46 ~~significant effect of condition on superficial temperature, where~~ Tail-docked~~D~~ cows had
47 ~~significantly~~ lower superficial temperature (26.43 ± 0.27 °C) ~~when~~ compared to ~~C~~control animals
48 ~~cows~~ (29.86 ± 0.62 °C).

49 **Discussion.** Pressure pain threshold values in both groups of animals were higher than~~t~~ those
50 previously reported for ~~tail-docked~~TD pigs, sows and cows. In contrast, pinprick stimulation
51 evaluates the presence of punctate mechanical hyperalgesia/allodynia, usually related to traumatic
52 nerve injury, and this association may reveal that it is possible that these animals developed a
53 disorder associated to the development of a tail stump neuroma and concurrent neuropathic pain,
54 previously reported in ~~tail-docked~~TD lambs, pigs and dogs. Thermal sensitivity showed that ~~tail-~~
55 ~~docked~~TD cows responded positively only to heat stimulation. These findings suggest that long-
56 term ~~tail-docked~~TD cows could be suffering hyperalgesia/allodynia, which may be indicative of
57 chronic pain. Lower superficial temperature in the stump may be associated to sympathetic fiber

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58 sprouting in the distal stump, which can lead to vasoconstriction and lower surface temperatures.

59 Further studies are needed in order to confirm neuroma development and adrenergic sprouting.

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61 Key Words: dairy cow, pain, tail-dockinged

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63 **Introduction**

64 Tail docking of dairy cows negatively impacts animal welfare (Stull et al., 2002). It comprises the

65 removal of a part of the tail and is usually performed by applying a rubber or latex ring a few

66 centimeters distal to the ventral aspect of the vulva (Petrie et al., 1996). Nowadays, tail docking is

67 considered a controversial practice with few studies reporting improvements in udder and milk

68 hygiene, cleaner cows by reducing the exposure to manure and mud and promoting personnel

69 comfort during the milking process (Schreiner & Ruegg, 2002a; Stull et al., 2002; Aubry, 2005).

70 In contrary, several reports have evaluated indirect measures of animal welfare and have found

71 that docked cows have increased fly loads leading to alterations of eating patterns resulting in a

72 decrease in milk production and increased fly avoidance behavior (Phipps, Matthews & Verkerk,

73 1995; Eicher et al., 2001) and restless behavior, including an increase in dorsal and lateral tail

74 stump movements (Eicher & Dailey, 2002; Tom et al., 2002; Eicher et al., 2006). Similarly, other

75 studies have not found differences in animal cleanliness, milk quality and somatic cell count

76 between docked and non-docked animals (Tucker, Fraser & Weary, 2001; Schreiner & Ruegg,

77 2002a).

78 Tail docking is prohibited in countries like Denmark, Germany, Sweden, Scotland, England,

79 Wales, and several states in the United States and Australia (Hepple & Clark, 2011; AVA, 2012;

80 AVMA, 2013). Similarly, the American and Canadian Veterinary Medical Associations strongly

81 oppose routine tail docking of cattle for management purposes (CVMA, 2016; AVMA, 2013). In
82 Chile, although not currently forbidden, a marked decrease in its practice has been observed.
83 Chilean legislation only indicates that painful procedures, such as tail docking should be performed
84 in a manner that minimizes pain and suffering (Chile, 2013).

85 Several reports indicate that tail docking results in few behavioral and physiological signs of acute
86 pain and distress in mature cows (Petrie et al., 1996; Eicher et al., 2000; Tom et al., 2002). Today,
87 veterinarians and general public accept the notion that chronic pain is different from acute pain
88 (Reichling & Levine, 2009); nonetheless, the uncertainty of whether acute pain can lead to the
89 development of chronic pain still exists (Voscopoulos & Lema, 2010). -According to Flecknell
90 (2008), the inconsistency of pain relief in cattle is the inadequate ability to assess pain. Chronic
91 pain assessment has not been investigated thoroughly in cattle, but castration and tail docking may
92 be associated to the development of chronic pain (Molony & Kent, 1997; Eicher et al., 2006).

93 According to Kroll et al. (2014) there is an increased risk for potential chronic pain development
94 at the amputation site, which has not been evaluated thoroughly in cows from commercial dairy
95 farms. Quantitative sensory testing (QST) is usually performed in order to diagnose chronic pain
96 conditions (Cruz-Almeida et al., 2014). It includes different methods that allow a characterization
97 of somatosensory function or dysfunction. The most common modalities-methods include thermal
98 (heat, cold) and mechanical (tactile, pressure) stimulation in order to elicit a painful or nonpainful
99 response (Fillingim et al., 2016). In addition, skin temperature evaluation can help determine tissue
100 metabolism and blood circulation; therefore, changes could reflect circulatory or inflammatory
101 conditions associated to chronic pain (Sathiyabarathi et al., 2016).

102 The ~~aims-objectives~~ of this study were first to evaluate the response to mechanical and thermal
103 stimulation, and second to determine the superficial temperature of the stump of tail-docked (TD)
104 dairy cows.

106 **Materials & Methods**

107 *Animals and housing*

108 This study was conducted between November and December 2015 on a commercial farm located
109 in Los Rios Region, southern Chile. The study was approved by the Ethics and Bioethics
110 Committee of Animal Research of the Universidad Austral de Chile (MV.21.2015). A total of 164
111 Holstein dairy cows with a mean age of 6.2 ± 1.94 years (parity range: 3-4), mean body weight of
112 423 ± 26 kg, mean milk yield of 27.3 ± 5.4 L day⁻¹ were enrolled. Only cows without clinical signs
113 of systemic disease during the last 15 days were selected. All evaluated cows were housed
114 individually in a tie-stall, fed a total mixed ration (TMR) and milked three times a day during the
115 entire period of study. From these, 133 cows were assigned to the ~~tail-docked TD group (TD)~~ and
116 31 cows were selected as control (C) animals (↔) and identified using the ear tag farm number.
117 Individual register showed that cows in the TD group were tail-docked at a mean age of 11.9 month
118 ~~of age~~ (range = 11.7-12.4) using a rubber band at a distance of approximately 10 cm below the
119 vulva by the farm veterinarian.

121 *Study design*

122 A clinical quantitative sensory assessment protocol was developed in order to evaluate the
123 presence of pain in ~~tail-docked TD~~ cows. Prior to sensory testing, cows were habituated to the
124 presence of the evaluator and experimental testing was performed during three consecutive days.

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125 After the morning milking, cows were allowed to return to their tie-stall individual cubicles and
126 were restrained using a headlock self-locking system for sensory assessment. The same evaluator
127 (RT) performed all the sensory assessments with the assistance of another researcher in charge of
128 identifying and recording positive reactions to the sensory stimuli (Eicher et al., 2006). In order to
129 avoid stress in the animals, both researchers approached the animals in a calm and quiet manner.

130 *Sensory assessments*

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

133 Pressure pain sensitivity: Sensitivity of the tail region in both groups of animals was evaluated
134 using a portable algometer (Wagner FDX 25 Compact Digital Force Gauge, Wagner Instruments,
135 Riverside, CT, USA) with a 1 cm² rubber probe. For each evaluation, the probe was constantly
136 applied in the same topographical location and placed perpendicular to the skin. The amount of
137 pressure applied during each evaluation was constantly increased at 500 grams of force *per second*
138 in the ventral surface of the tail stump (TD), and ventral surface of the tail (C), respectively, until
139 a positive response was obtained. Each area was assessed five times at 60-second intervals. Lateral
140 and ventral movement and/or withdrawal of the tail were considered positive responses, in which
141 the pressure elicited by the algometer was immediately discontinued and pressure registered. The
142 mean of 5 measurements per site was considered as a single value per tested cow.

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

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

152 *Superficial temperature*

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

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162 *Statistical analysis*

163 For each continuous variable, probability plots were generated to verify that data followed a
164 normal distribution. Pressure pain threshold and superficial temperature were analyzed using
165 analysis of covariance. The adjusted linear model included condition (TD versus C) as fixed effect
166 and age as covariate. Body weight, ~~number of~~ parity and milk yield ~~did not have a significant~~
167 ~~effect on~~ were not associated with -pressure pain threshold and superficial temperature, and thus
168 were removed from the model ~~from the model~~ using a backward selection process. For pinprick
169 and thermal sensitivity, Pearson's Chi-~~s~~Square with Yates' continuity correction tests were
170 conducted to analyze for possible association between condition and sensitive stimulation. For all

171 statistical procedures, the overall alpha was set to 0.05. The statistical analysis was performed
172 using R Statistical Software (R Core Team, Vienna, Austria).

173

174 Results

175 ~~Lower p~~Analysis revealed a significant effect of condition (TD versus C) on the pressure pain
176 threshold ~~values were~~ ($P < 0.001$, $\eta_p^2 = 0.46$) with significantly lower values necessary to obtain a
177 withdrawal response in TD cows (5.97 ± 0.19 kg) compared to C cows (11.75 ± 0.43 kg) ($P < 0.001$,
178 $\eta_p^2 = 0.46$) (Fig. 1). ~~Condition (TD versus C) Chi Square test revealed that there was a significant~~
179 ~~association between with~~ heat sensitivity ~~and condition~~ ($\chi^2 = 10.36$, $df = 1$, $P = 0.0013$) with 29.3%
180 of TD cows responding positively (Table 1). ~~Nonetheless~~In contrast, no association was ~~found~~
181 ~~present~~ between condition and cold sensitivity ($\chi^2 = 3.46$, $df = 1$, $P = 0.062$) (Table 1). Similarly, after
182 pinprick sensitivity test was performed, 93.2% of TD cows elicited a positive response to
183 stimulation. This sensory testing was ~~significantly~~ associated with the condition ($\chi^2 = 7.87$, $df = 1$,
184 $P = 0.005$). ~~There was a significant effect of condition on superficial temperature~~ ($P < 0.001$, $\eta_p^2 = 0.13$),
185 ~~where~~ TD cows had ~~significantly~~ ($P < 0.001$) lower ~~superficial~~ temperature (26.43 ± 0.27
186 °C) ~~when~~ compared to C ~~animals~~ cows (29.86 ± 0.62 °C) ($P < 0.001$, $\eta_p^2 = 0.13$) (Fig. 2).

187

188 Discussion

189 Painful procedures are performed in the dairy industry and they are often associated with the
190 development of fear, distress and chronic pain of animals (Grandin, 2015). Tail docking is a painful
191 procedure that induces both acute and chronic pain, and leads to behavioral modifications and
192 discomfort (Tucker, Fraser & Weary 2001). Different studies have confirmed the presence of acute

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193 pain and augmented animal activity, characterized by a marked increase in foot stomp [behaviors](#)
194 following tail docking (Eicher & Dailey 2002; Schreiner & Ruegg 2002b; Tom et al., 2002).

195 Tail-docked cows ~~showed significantly~~[had](#) lower pressure pain threshold compared to controls.
196 ~~The~~[se](#) results are similar to those reported in pigs, in which mechanical sensitization of the tail
197 stump lasted for up to 16 weeks (Di Giminiani et al., 2017). Pressure pain threshold values in both
198 groups of animals were higher ~~than~~[t](#) those previously reported for ~~tail-docked~~[TD](#) pigs (Di
199 Giminiani et al., 2016), sows (Nalon et al., 2016) and cows stimulated using an algometer with a
200 metal probe in the third metatarsal bone (Raundal et al., 2014). The higher overall pressure values
201 described in this study could be related to the use of a rubber probe. According to Di Giminiani et
202 al., (2016), the use of different probes could be associated to an increased degree of response
203 variability. Similarly, Taylor and Dixon (2012) mention that an increase in probe diameter results
204 in higher variability. Other factors that may influence the higher values of pressure threshold
205 presented here may include skin thickness (Di Giminiani et al., 2016), individual variation (Nalon
206 et al., 2016), and stress-induced hypoalgesia (Herskin, Munksgaard & Ladewig, 2004).

207 The ~~significant~~ association between condition and heat sensitivity is similar to that reported by
208 Eicher et al., (2006), in which ~~tail-docked~~[TD](#) cows manifested ~~less~~[er number of](#) foot stomps, foot
209 shifts and tail swings. ~~Nonetheless, in this study we did not find an significant association (P=~~
210 ~~0.0629)~~ between condition and cold sensitivity. Moreover, here we report a significant association
211 between condition and pinprick sensitivity. Impaired sensitivity to pinprick has been previously
212 reported in amputated human patients (Kosasih & Silver-Thorn, 1998). Pinprick stimulation
213 evaluates the presence of punctate mechanical hyperalgesia/allodynia, usually related to traumatic
214 nerve injury (Jensen & Finnerup, 2014). This association suggests that animals may have
215 developed tail stump neuroma as reported previously in other species. Petrie et al., (1996) indicate

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216 that tail docking would induce tissue damage that leads to neuromata development and concurrent
217 neuropathic pain. Moreover, neuroma development has been previously reported in tail-docked
218 lambs (French & Morgan, 1992; Fisher & Gregory, 2007), pigs (Herskin, Thodberg & Jensen,
219 2015; Kells et al., 2017) and dogs (Gross & Carr, 1990). Peripheral neuromas occur in 10-25% of
220 human amputees, and are generally formed after injury or surgical procedures, resulting in
221 neuropathic pain, residual limb pain, functional impairment and psychological distress (Rajput,
222 Reddy & Shankar, 2012), increasing sensitivity to mechanical and thermal stimulation (Toia et al.,
223 2015; O'Reilly et al., 2016; Yao et al., 2017). -Histopathological analysis confirmed the presence
224 of neuroma in the tail stump of docked pigs one month after tail docking, characterized by marked
225 nerve sheath and axonal proliferation (Sandercock et al., 2016). Moreover, another study in pigs
226 identified age at time of the procedure as a factor that may influence the development of
227 neuropathic pain (Di Giminiani et al., 2017). Nonetheless, cows in the present study were, on
228 average tail docked 48 months before sensory evaluation. According to this, we believe that pain
229 experienced by docked cows is similar to human phantom limb pain (Nikolajsen, 2012). In this
230 condition, the amputation and trauma that nerves and surrounding tissue suffer, disrupts normal
231 afferent and efferent signals, leads to neuroma development and neurons become hyper-excitabile
232 (Hanyu-Deutmeyer and Dulebohn, 2018). Phantom limb pain has been vastly studied in humans
233 (Schley et al., 2008; Andoh et al., 2017; Yin et al., 2017). In cases of phantom limb pain,
234 characteristic chronic neuropathic pain occurs in the amputation stump; and although this pain may
235 decrease or eventually disappear over time, if it continues for more than 6 months, the prognosis
236 for pain decrease is poor (Kuffler, 2017).

237 Surface temperature was significantly lower in the TD group compared to controls. Similar results
238 were reported by Eicher et al. (2006), where the stump of docked heifers ~~were~~ was approximately

239 2°C ~~less-colder~~ than the underside of the tails of intact heifers. Similar results have been described
240 in amputated humans, in which the stump of amputated limbs ~~reflects-had~~ lower superficial
241 temperatures than the contralateral side using a temperature probe (Hunter, Kats & Davis, 2005)
242 and thermographic analysis (Harden et al., 2008). This decrease in temperature may be associated
243 ~~to-with~~ sympathetic fiber sprouting in the distal stump, which can lead to vasoconstriction and
244 lower surface temperatures (Harden et al., 2004). Similarly, Nascimento et al. (2015), after
245 traumatic nerve injury confirms the presence of sympathetic sprouting in the skin that contributes
246 to pain.

247 In this study, we showed evidence that may confirm the development of chronic pain states
248 (hyperalgesia/allodynia) in long term ~~tail-dockedTD~~ dairy cows. Some limitations of the study
249 include the ~~despair-unequal~~ number of animals in ~~each-the-two~~ experimental groups, ~~selection of~~
250 ~~tail-dockedTD cows~~ ~~and other factors such as breed, method, and intensive verses pasture system,~~
251 ~~climate, and nutrition that may influence painful condition in cowsand -under commercial~~
252 ~~conditionsweather conditions that may affect thermography.- Future research in tail-dockedTD~~
253 ~~cows must include the evaluation of other indicators of welfare such as milk production, motor~~
254 ~~activity and plasma biomarkers of pain and stress. Moreover, As is other species, itin order to~~
255 ~~confirm neuropathic pain-, neuroma formation in cows must be demonstrated using a thorough~~
256 ~~histopathological examinationwould be interesting to evaluate and confirm the presence of~~
257 ~~neuromas in cows, especially considering results from our study were they showed sensitivity.~~

258 Nonetheless, our results confirm that tail docking of dairy cows is a practice that affects animal
259 welfare (Stull et al., 2002).

260

261 **Conclusions**

Commented [HB10]: Be specific. What was a limitation in the selection of TD cows?

Commented [HB11]: Milk production is not a welfare indicator

262 ~~Results of the present study indicate that T~~tail-docked cows had an increased response to
263 mechanical stimulation characterized by lower pain pressure thresholds and a positive association
264 to pinprick sensitivity. Thermal sensitivity showed that ~~tail-docked~~TD cows responded positively
265 only to heat stimulation. These findings suggest that in the long-term ~~tail-docked~~TD cows could
266 ~~be~~ suffer ~~from~~ing hyperalgesia/allodynia, which may be indicative of chronic pain. Lower
267 superficial temperature in the stump, could be associated ~~to~~with adrenergic tissue sprouting
268 inducing peripheral vasoconstriction. Further studies are needed in order to confirm neuroma
269 development and adrenergic sprouting.

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