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

Renata J Troncoso [1] , **Daniel E Herzberg** [2] , **Constanza S Meneses** [2] , **Heine Y Müller** [2] , **Marianne P Werner** [Corresp., 3] , **Hedie Bustamante** [Corresp. 1]

[1] Veterinary Clinical Sciences Department, Universidad Austral de Chile, Valdivia, Chile
[2] Graduate School, School of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile
[3] Animal Science Department/School of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile

Corresponding Authors: Marianne P Werner, Hedie Bustamante
Email address: marianne.werner@uach.cl, hbustamante@uach.cl

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

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

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

Renata J. Bravo*, Daniel E. Herzberg‡, Constanza S. Meneses‡, Heine Y. Müller‡, Marianne P. Werner†, Hedie A. Bustamante*[1]

*Veterinary Clinical Sciences Department, School of Veterinary Sciences, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile

†Animal Science Department, School of Veterinary Sciences, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile

‡Graduate School, School of Veterinary Sciences, Universidad Austral de Chile, Campus Isla Teja, Valdivia, Chile

[1] Hedie A. Bustamante, Casilla 567 Valdivia, +56632221077, hbustamante@uach.cl

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:

<u>Pressure pain sensitivity</u>: 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.

<u>Thermal sensitivity</u>: Cold and heat sensitivity assessment was performed using a frozen pack (0ºC) and warm water (45ºC), 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 ºC) when compared to C animals (29.86±0.62 ºC) (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 that 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 2ºC 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.

References

Andoh J, Milde C, Tsao JW, Flor H. 2017. Cortical plasticity as a basis of phantom limb pain: Fact or fiction? *Neuroscience* DOI: 10.1016/j.neuroscience. 2017.11.015.

Aubry P. 2005. Routine surgical procedures in dairy cattle under field conditions: abomasal surgery, dehorning, and tail docking. *Veterinary Clinics Food Animals* 21:55-72.

Barnett JL, Coleman GJ, Hemsworth PH, Newman EA, Fewings-Hall S, Ziini C. 1999. Tail docking and beliefs about the practice in the Victorian dairy industry. *Australian Veterinary Journal* 77:742-747.

Chile. 2013. Ley Nº 20.380 Decreto 29 Sobre protección de los animals durante su producción industrial, su comercialización y en otros recintos de mantención de animales. Biblioteca del Congreso Nacional de Chile.

Di Giminiani P, Sandercock DA, Malcolm EM, Leach MC, Herskin MS, Edwards SA. 2016. Application of a handheld pressure application measurement device for the characterization of mechanical nociceptive threshold in intact pig tails. *Physiology & Behavior* 165: 119-126.

Di Giminiani P, Nasirahmadi A, Malcolm EM, Leach MC, Edwards SA. 2017. Docking piglet tails: how much does it hurt and for how long? *Physiology Behavior* 182: 69-76.

Eicher SD, Morrow-Tesch JL, Albright JL, Williams RE. 2001. Tail-docking alters fly numbers, fly-avoidance behaviors, and cleanliness, but not physiological measures. *Journal of Dairy Science* 84: 1822-1828.

Eicher SD, Dailey JW. 2002. Indicators of acute pain and fly avoidance behaviors in Holstein calves following tail-docking. *Journal of Dairy Science* 85: 2850-2858.

Eicher SD, Cheng HW, Sorrells AD, Schutz MM. 2006. Behavioral and physiological indicators of sensitivity or chronic pain following tail docking. *Journal of Dairy Science* 89: 3047-3051.

Elliott REW. 1969. The effect of tail amputation on the milk yield of cows. Letter to the Editor. *New Zealand Veterinary J*ournal 17: 89.

Fischer MW, Gregory NG. 2007. Reconciling the difference between the length at which lambs´ tail are commonly docked and animal welfare recommendations. *Proceedings of the New Zealand Society of Animal Production* 67: 32-38.

Fitzgerald M, McKelvey R. 2016. Nerve injury and neuropathic pain – A question of age. *Experimental Neurology* 275: 296-302.

French NP, Morgan KL. 1992. Neuromata in docked lambs´ tails. *Research in Veterinary Science* 52: 389-390.

Fulwider WK, Grandin T, Rollin BE, Engle TE, Dalsted NL, Lamm WD. 2008. Survey of dairy management practices on one hundred thirteen North Central and Northeastern United States dairies. *Journal of Dairy Science* 91: 1686-1692.

Grandin T. 2015. The importance of measurement to improve the welfare of livestock, poultry, and fish. In: Grandin T, ed. *Improving Animal Welfare* 2nd Ed., Oxfordshire: CAB International, 15-34.

Gross TL, Carr SH. 1990. Amputation neuroma of docked tails in dogs. *Veterinary Pathology* 27: 61-62.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

Guatteo R, Levionnois O, Fournier D, Guémené D, Latouche K, Leterrier C, Mormède P, Prunier A, Servière J, Terlouw C, Le Neindre P. 2012. Minimizing pain in farm animals: the 3S approach - ¨Suppress, Substitute, Soothe¨. *Animal* 6: 1261-1274.

Harden RN, Rudin N, Bruehl S, Parikh D, Kooch J, Gracely R. 2004. Elevated systemic catecholamines in complex regional pain syndrome and relationship to psychological factors: a pilot study. *Anesthesia and Analgesia* 99: 1478-1485.

Harden RN, Gagnon CM, Gallizzi M, Khan AS, Newman D. 2008. Residual limbs of amputees are significantly cooler than contralateral intact limbs. *Pain Practice* 8: 342-347.

Hepple S, Clark J. 2011. Tail docking of dairy cattle in the UK. Letters. *Veterinary Record* DOI: 10.1136/vr.d2960.

Herskin MS, Munksgaard L, Ladewig J. 2004. Effects of acute stressors on nociception, adrenocortical responses and behavior of dairy cows. *Physiology and Behavior* 83: 411-420.

Herskin MS, Thodberg K, Jensen HE. 2015. Effects of tail docking and docking length on neuroanatomical changes in healed tail tips of pigs. *Animal* 9: 677-681.

Hunter JP, Katz J, Davis KD. 2005. Dissociation of phantom limb phenomena from stump tactile spatial acuity and sensory threshold. *Brain* 128: 308-320.

Jensen TS, Finnerup NB. 2014. Allodynia and hyperalgesia in neuropathic pain: clinical manifestation and mechanisms. *The Lancet. Neurology* 13: 924-935.

Kells NJ, Beausoleil NJ, Johnson CB, Sutherland MA, Morrison RS, Roe W. 2017. Comparison of neural histomorphology in tail tips from pigs docked using clippers or cautery iron. *Animal* 11: 1222-1227.

Kosasih JB, Silver-Thorn MB. 1998. Sensory changes in adults with unilateral transtibial amputation. *Journal of Rehabilitation Research and Development* 35: 85-90.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

Kroll LK, Grooms DL, Siegford JM, Schweihofer JP, Daigle CL, Metz K, Ladoni M. 2014. Effects of tail docking in behavior of confined feedlot cattle. *Journal of Animal Science* 92: 4701-4710.

Kuffler DP. 2017. Origins of phantom limb pain. *Molecular Neurobiology* 55: 60-69.

Nalon E, Maes D, Piepers S, Taylor P, van Riet MJM, Janssens GPJ, Millet S, Tuyttens FAM. 2016. Factors affecting mechanical nociceptive thresholds in healthy sows. *Veterinary Anaesthesia and Alagesia* 43: 343-355.

Nascimento FP, Magnussen C, Yousefpour N, Ribeiro-da-Silva A. 2015. Sympathetic fibre sprouting in the skin contributes to pain-related behaviour in spared nerve injury and cuff models of neuropathic pain. *Molecular Pain* DOI: 10.1186/s12990-015-0062-x.

Nikolajsen L. 2012. Postamputation pain: studies on mechanisms. *Danish Medical Journal* 59: B4527.

O´Reilly MAR, O´Reilly PMR, Sheahan JN, Sullivan J, O´Reilly HM, O´Reilly MJ. 2016. Neuromas as the cause of pain in the residual limbs of amputees. An ultrasound study. *Clinical Radiology* 71: 1068.e1-1068.e6.

Petrie NJ, Mellor DJ, Stafford KJ, Bruce RA, Ward RN. 1996. Cortisol response of calves to two methods of tail docking used with or without local anaesthetic. *New Zealand Veterinary Journal* 44: 4-8.

Phipps AM, Matthews LR, Verkerk GA. 1995. Tail docking dairy cattle: fly induced behaviour and adrenal responsiveness to ACTH. *Proceedings of the New Zealand Society of Animal Production* 55: 61-63.

Rajput K, Reddy S, Shankar H. 2012. Painful neuromas. The *Clinical Journal of Pain* 28: 639-645.

Raundal PM, Andersen PH, Toft N, Forkman B, Munksgaard L, Herskin MS. 2014. Handheld mechanical nociceptive threshold testing in dairy cows-intra-individual variation, inter-observer agreement and variation over time. *Veterinary Anaesthesia and Analgesia* 41: 660-669.

Sandercock DA, Smith SH, Di Giminiani P, Edwards SA. 2016. Histopathological characterization of tail injury and traumatic neuroma development after tail docking in piglets. *Journal of Comparative Pathology* 155: 40-49.

Schley MT, Wilms P, Toepfner S, Schaller HP, Schmelz M, Konrad CJ, Birbaumer N. 2008. Painful and nonpainful phantom and stump sensations in acute traumatic amputees. *The Journal of Trauma* 65: 858-864.

Schreiner DA, Ruegg PL. 2002a. Effects of tail docking on milk quality and cow cleanliness. *Journal of Dairy Science* 85: 2503-2511.

Schreiner DA, Ruegg PL. 2002b. Responses to tail docking in calves and heifers. *Journal of Dairy Science* 85: 3287-3296.

Stull CL, Payne MA, Berry SL, Hullinger PJ. 2002. Evaluation of the scientific justification for tail docking in dairy cattle. *Journal of the American Veterinary Medical Association* 220: 1298-1303.

Sutherland MA, Tucker CB. 2011. The long and short of it: A review of tail docking in farm animals. *Applied Animal Behaviour Science* 135: 179-191.

Taylor PM, Dixon MJ. 2012. The relationship between tip diameter and mechanical nociceptive threshold. *Proceedings of the 14th World Congress on Pain, Non-human species-special ntrest group.* Milan, Italy, p 3.

Toia F, Giesen T, Giovanoli P, Calcagni M. 2015. A systematic review of animal models for experimental neuroma. *Journal of Plastic, Reconstructive & Aesthetic Surgery* 68: 1447-1463.

Tom EM, Duncan IJH, Widowski TM, Bateman KG, Leslie KE. 2002. Effects of tail docking using a rubber ring with or without anesthetic on behavior and production of lactating cows. *Journal of Dairy Science* 85: 2257-2265.

Tucker CB, Fraser D, Weary DM. 2001. Tail docking dairy cattle: Effects on cow cleanliness and udder health. *Journal of Dairy Science* 84: 84-87.

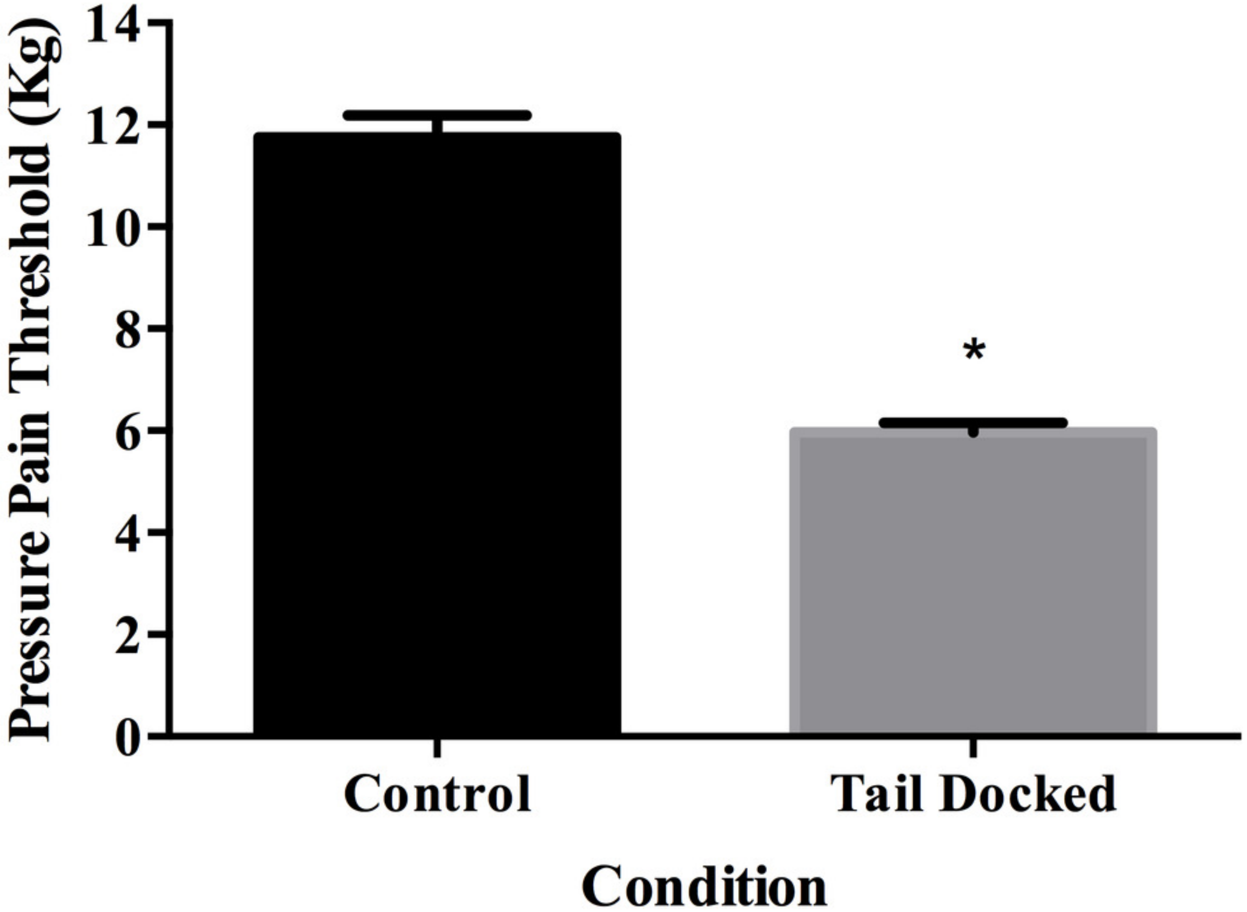
Yao C, Zhou X, Zhao B, Sun C, Poonit K, Yan H. 2017. Treatments of traumatic neuropathic pain: a systematic review. *Oncotarget* 8: 57670-57679.

Yin Y, Zhang L, Xiao H, Wen CB, Dai YE, Yang G, Zuo YX, Liu J. 2017. The pre-amputation pain and the postoperative deafferentation are the risk factors of phantom limp pain: a clinical survey in a sample of Chinese population. *BMC Anesthesiology* DOI: 10.1186/s12871-017-0359-6.

PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

Figure 1

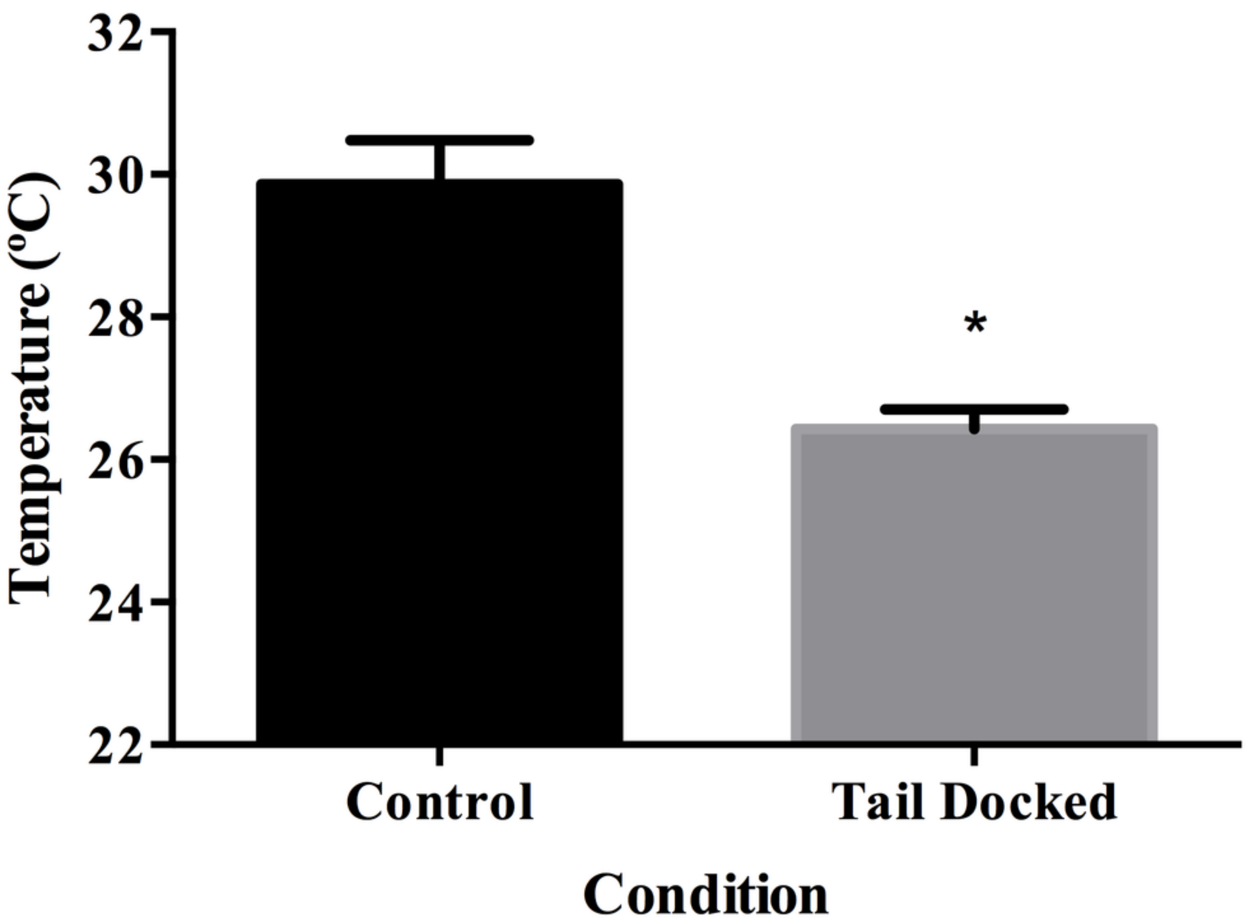
Least square means and standard error for pressure pain threshold in tail docked (n=133) and control cows (n=31). *Statistically significant differences between groups (P<0.001).



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

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).



PeerJ Preprints | https://doi.org/10.7287/peerj.preprints.26550v1 | CC BY 4.0 Open Access | rec: 20 Feb 2018, publ: 20 Feb 2018

Table 1 (on next page)

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).

	Tail docked		Control		P value
	Positive N (%)	Negative N (%)	Positive N (%)	Negative N (%)	
Heat sensitivity	39 (29.3)	94 (70.7)	0 (0)	31 (100)	0.0013
Cold sensitivity	31 (23.3)	102 (76.6)	2 (6.6)	29 (93.6)	0.0629
Pinprick stimulus	124 (93.2)	9 (6.8)	23 (74.2)	8 (26.9)	0.005

*P values for Chi square test