

Plasma concentration of norepinephrine, β -endorphin, and substance P in lame dairy cows

Alfredo Rosamel Rodríguez¹, Daniel Eduardo Herzberg², Marianne Patricia Werner⁴, Heine Yacob Müller², Hedio Almagro Bustamante³

¹Agricultural Sciences Graduate School, ²Veterinary Sciences Graduate School, ³Veterinary Clinical Sciences Department, ⁴Animal Science Department, Universidad Austral de Chile, 5110626, Valdivia, Chile, hbustamante@uach.cl

Received: February 21, 2018 Accepted: June 13, 2018

Abstract

Introduction: Lameness is a painful and debilitating condition that affects dairy cows worldwide. The aim of this study was to determine the plasma concentration of norepinephrine, β -endorphin, and substance P in dairy cows with lameness and different mobility scores (MS). **Material and Methods:** A total of 100 Friesian and Jersey cows with lameness (parity range: 1–6; weight: 400–500 kg; milk yield: 22–28 L a day, and lactation stage less than 230 days) were selected. Animals were selected and grouped according to MS (MS 0–3; n = 25), and plasma concentration of norepinephrine, substance P, and β -endorphin was measured using ELISA. **Results:** Cows with MS 3 had higher plasma concentrations of norepinephrine and substance P and lower plasma concentrations of β -endorphins when compared to MS 0 cows. **Conclusion:** Variations in plasma concentration of norepinephrine, substance P, and β -endorphin could be associated with intense pain states in dairy cows with lameness, but are insufficient to differentiate these states from the mildest pain states. Further studies are necessary in order to evaluate the potential use of these biomarkers in the detection of chronic bovine painful conditions.

Keywords: dairy cows, norepinephrine, β -endorphin, substance P, lameness.

Introduction

Lameness is a debilitating condition that affects dairy cow health, behaviour, and welfare (26), decreases milk production and fertility, and increases treatment costs and premature culling. Although its aetiology is not fully understood, it is well known that lameness is a multifactorial condition with potential causes such as trauma, metabolic disorders, and infections (4, 34). In terms of duration and evolution, lameness can be considered a chronic condition inducing a decrease in the nociceptive threshold (hyperalgesia) of the affected limb lasting more than 28 days, suggesting chronic pain (33). Recently, the use of biomarkers, defined as measurable indicators of normal biological processes, pathogenic processes, or therapeutic responses, has spread from the laboratory to clinical practice (3). The use of blood biomarkers could predict risk of pain, potentially improving our diagnostic approach to painful conditions such as

lameness (5). Although, it is broadly accepted that pain triggers a stress-related autonomic response, the neuroendocrine mechanisms underlying the relationship between pain and stress are not completely elucidated (14). The function of the autonomic nervous system (ANS) and particularly the plasma concentration of norepinephrine (NE) have been extensively used as biomarkers of stress and pain (6). β -endorphin (BE) is an endogenous anti-nociceptive neuropeptide and an important pain biomarker only reported in pregnant cows (23) and cattle during slaughter (30). Substance P (SP) is a pro-nociceptive neuropeptide that regulates dorsal horn neuron excitability after injury or during intense pain conditions (8). Painful procedures such as castration and dehorning of calves have been shown to increase SP plasma concentration (9, 10). Moreover, evidence has shown that SP may play a role in the development of inflammatory conditions such as arthritis in humans (28).

The aim of the present study was to determine the plasma concentrations of NE, BE, and SP in dairy cows with lameness and different mobility scores.

Material and Methods

Between June 2013 and January 2014, a total of 9,000 multiparous lactating dairy cows were evaluated for lameness. They were kept on 17 commercial farms based on the grazing system located in Southern Chile. The herds were selected by convenience based on the disposition of the farm manager to participate in the study. During each farm visit, all lactating cows were observed at the same time of the day with the purpose of detecting animals with evident lameness, and only those with hind limb lameness were included. As an inclusion criterion, only animals with diagnosed lameness lasting less than 60 days were selected. A trained observer performed mobility scoring immediately after the cows left the parlour using the scale described by Reader *et al.* (27). Briefly, cows were mobility scored as follow: MS 0 (not lame) indicated good mobility/sound; MS 1 demonstrated imperfect mobility/uneven; MS 2 revealed impaired mobility/mildly lame; and MS 3 indicated severely impaired mobility/very lame. After evaluation and selection of animals, a total of 100 Holstein Friesian (55) and Jersey (45) cows with a parity range of 1–6, live weight range of 400–500 kg, milk yield of 22–28 L a day, and lactation stage less than 230 days were enrolled in the study. Control animals (MS 0) were randomly selected using the same mobility scoring system. Once this selection was performed, cows were distributed into four groups: MS 0 (n = 25), MS 1 (n = 25), MS 2 (n = 25), and MS 3 (n = 25).

During the last follow up visit, and after confirmation of MS, each animal was restrained in a crush in order to perform clinical examination, and only those animals were included with no evidence of

disease or health disorder and not under analgesic treatment. Blood samples (10 mL) were collected to heparinised tubes from the mammary vein and refrigerated at 4°C until their processing. After centrifugation (1,500 × g, 15 min, 4°C) plasma was obtained, aliquoted into 1.5 mL tubes, and frozen at –70°C for biochemical analysis. NE concentrations were measured using a sandwich ELISA validated for bovine plasma (Cusabio Biotech Co., USA). The intra- and inter-assay coefficient of variation (CV) was 15%. BE concentrations were measured using a sandwich ELISA validated for bovine plasma (Cusabio Biotech Co., USA) and in the same way SP concentrations were also measured. The intra- and inter-assay CVs were 8% and 10%, respectively.

For each outcome variable, probability and residual plots were generated in order to verify that data followed a normal distribution. Linear mixed models were fitted using each biomarker as outcome variable and MS as fixed effect, including the farm as a random effect. Confidence intervals not including zero indicated statistical significance using Wald's statistics with a P value <0.05. Statistical analysis was performed using RStudio (RStudio, USA).

Results

Total presentation frequencies of foot lesions for each mobility score (Table 1) were 33.1%, 33.2%, and 33.3% for MS 1, MS 2, and MS 3, respectively. The most frequently diagnosed lesions for MS 1, MS 2, and MS 3 were white line disease (10%), sole haemorrhage (12.2%), and ulcer (7.8%), respectively. Considering the origin of the lesion, 87.9% of the cows had noninfectious lesions (*e.g.* white line disease, sole haemorrhage, ulcer, etc.) while 12.1% had infectious lesions (*e.g.* heel erosion or digital and interdigital dermatitis).

Table 1. Frequency of occurrence of the main foot lesions diagnosed (n = 75; 25 per MS group)

Claw lesions diagnosed	MS 1 (%)	MS 2 (%)	MS 3 (%)	Total (%)
Infectious lesion				
Digital dermatitis	0	0	3.3	3.3
Heel erosion	2.2	2.2	0	4.4
Interdigital dermatitis	1.1	0	3.3	4.4
Non-infectious lesion				
Abscess	4.4	1.1	6.7	12.2
Double sole	4.4	2.2	1.1	7.8
Heel inflammation	3.3	0	1.1	4.4
Interdigital callus	0	0	2.2	2.2
Overgrowth	1.1	1.1	0	2.2
Sole haemorrhage	4.4	12.2	2.2	18.9
Ulcer	2.2	3.3	7.8	13.3
White line disease	10	11.1	5.6	26.7

The mean plasma concentrations of NE in MS 0 and MS 1 cows were 655.83 ± 24.09 pg/mL and 650.97 ± 35.54 pg/mL, respectively, with no statistical significant difference between these concentrations (Fig. 1a). Cows with an MS 2 score showed an insignificant mean increase of 65.85 ± 34.07 pg/mL compared to cows with MS 0 scores (Table 2). In contrast, cows scored at MS 3 showed a statistically significant mean increase of 245.45 ± 33.66 pg/mL (Fig. 1a) compared to cows scored at MS 0 ($P = 0.0000000279$) (Table 2). The mean plasma concentration of BE decreased in all groups compared to cows assessed at MS 0. Plasma concentrations of BE in MS 0 and MS 1 cows were 46.60 ± 1.49 pg/mL and

42.8 ± 2.20 pg/mL, with no statistically significant difference between these concentrations (Fig. 1b). Accordingly, cows with MS 2 ($P = 0.008093$) and MS 3 ($P = 0.000342$) had mean decreases in plasma BE of 5.75 ± 2.11 pg/mL and 7.84 ± 2.09 pg/mL, respectively, compared to cows with MS0 (Table 2). The mean plasma concentration of SP increased linearly in MS groups as MS score rose. Cows in the MS 0, MS 1, and MS 2 groups had mean values of 0.25 ± 0.09 ng/mL, 0.32 ± 0.13 ng/mL, and 0.42 ± 0.12 ng/mL (Fig. 1c). In contrast, cows in the MS 3 group showed a significant mean increase of 0.61 ± 0.12 ng/mL compared to MS 0 cows ($P = 0.000043$) (Table 2).

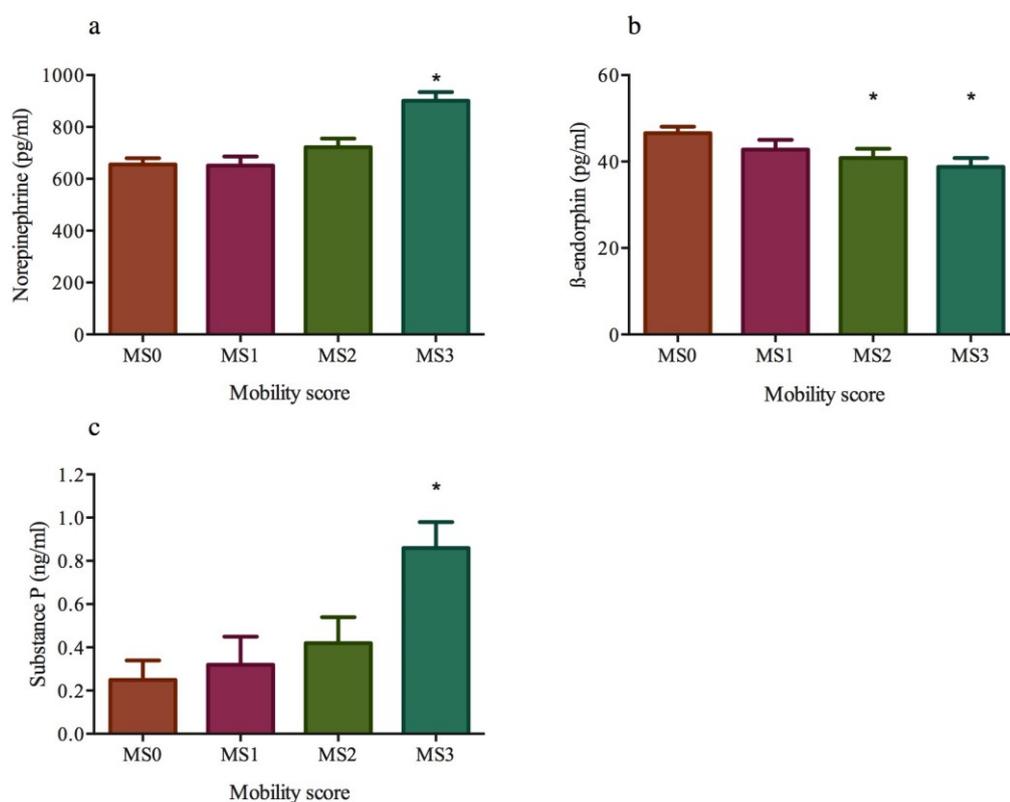


Fig. 1. Mean (+SD) plasma concentration of norepinephrine (a), β -endorphin (b), and substance P (c) of chronically lame and control dairy cows ($N = 25$ per MS). * – significant differences compared to MS 0, $P < 0.05$

Table 2. Linear mixed effect model of mobility scoring (MS) on norepinephrine, β -endorphin, and substance P plasma concentrations in lame and control dairy cows ($N = 25$ per MS)

Variable	Estimated	Std. Error	95% CI
Norepinephrine			
MS0	655.83	24.09	607.83 – 703.83
MS1	-4.86	35.54	-75.67 – 65.94
MS2	65.85	34.07	-2.03 – 133.72
MS3	245.45	33.66	178.39 – 312.51
β-endorphin			
MS0	46.6	1.49	43.61 – 49.57
MS1	-3.8	2.2	-8.20 – 0.59
MS2	-5.75	2.11	-9.96 – -1.54
MS3	-7.84	2.09	-11.0 – -3.68
Substance P			
MS0	0.25	0.09	0.08 – 0.43
MS1	0.07	0.13	-0.19 – 0.33
MS2	0.17	0.12	-0.08 – 0.41
MS3	0.61	0.12	0.37 – 0.86

Confidence intervals not including zero denote significant effect compared to MS0

Discussion

The aim of the present study was to determine the plasma concentration of NE, BE, and SP in dairy cows with lameness and different mobility scores. We hypothesised that lameness in dairy cows is a painful condition that leads to an increase in the plasma concentration of the compounds. The main finding in our study is that cows with intense lameness-associated pain reflected by their mobility score (MS 3) had a higher plasma concentration of NE and SP, and lower plasma concentration of BE when compared to MS 0 cows. Plasma concentrations of NE in sound cows (MS 0) were similar to those previously reported in dairy heifers (6). Nonetheless, cows with severe lameness (MS 3) showed an increase in plasma NE concentration, which is concordant with previous findings in chronically lame ewes and cattle (18, 19). Previous findings indicate that NE plasma concentration increases between 5 and 30 min post acutely painful and stressful procedures such as branding (16), weaning (17), and castration (31). Comparably, Bustamante *et al.* (6) reported an increase in plasma NE concentration 6 h after induction of acute lameness using an oligofructose overload model in dairy heifers. The finding here reported could be associated with the activation of the sympathetic nervous system (SNS), which increases its activity during pain states. Similarly, Tsigos *et al.* (32) described elevated levels of circulating NE in human patients with chronic pain, suggesting a higher number of functioning sympathetic fibres. According to Banik *et al.* (2), in chronically injured peripheral tissue, this noradrenergic mechanism may lead to neuronal modifications such as sprouting of postganglionic sympathetic nerve fibres or up- or down-regulation of adrenoreceptor subtypes, which may lead to an increase in excitability of primary afferent nociceptors aggravating pain perception. Also, recent studies indicate that chronic pain conditions induce an increase in plasma NE concentration that may be associated with a deregulation of the supraspinal descending inhibitory pathway (24). Additionally, plasmatic NE concentrations could also be related to blood pressure regulation, which is known to be important during endogenous pain control (25). Similarly, it has been previously reported that lame cows significantly modify their behaviour, decreasing their activity, and feeding and social patterns (13). These important changes could lead to homeostatic modification, particularly in hormone secretion that may affect the ANS response.

Plasma BE concentration was similar to those reported for sound cows (6). Our results showed a significant reduction in plasma BE in cows with MS 3 compared to cows with MS 0 (Fig. 1b). During an acute nociceptive insult endogenous opioids act as the main antinociceptive system (11). After oligofructose overload-induced lameness Bustamante *et al.* (6)

described an acute increase in BE plasma concentration reflecting the activation of a well-integrated nociceptive/antinociceptive pathway. The potential role of endogenous opioids in the transition between acute and chronic pain has recently been a subject of debate and a dysfunction or loss in the antinociceptive properties of the endogenous opioid system during chronic pain states has been described (7). In contrast, Doehring *et al.* (12) showed that chronic opioid exposure correlated with an increase in pain perception in humans. Chronic opioid exposure can stimulate nociceptive transmission at spinal levels (21) and activate a glial response (15).

Substance P plasma concentration increased with lameness, showing statistical significant differences between MS 3 and MS 0 cows. Interestingly, SP plasma concentration from cows with MS 0 was similar to those previously reported for sound cattle (6). SP is a well-defined mediator of acute pain in cattle during acute lameness (6), castration (9), and dehorning (1). Recently, its potential role in chronic pain conditions like osteoarthritis and chronic inflammatory state has been reported (20). Pro-inflammatory cytokines can increase SP synthesis from primary afferent nociceptors and by this mechanism decrease nociceptive stimulation threshold during acute pain (22). Moreover, the activation of NK1 receptors by SP can induce glial activation and a deregulation in the synaptic homeostasis between primary afferent nociceptors and pain projection neurons in the dorsal horn, leading to the development of chronic pain states (29). The increase in plasma SP in cows with MS 3 (Fig. 1c) may confirm its role as pain facilitator in chronic pain states.

We conclude that variations in the plasma concentration of NE, SP, and BE could be associated with intense pain states in dairy cows with lameness, but are insufficient to differentiate these states from the mildest pain states. Further studies are necessary in order to evaluate the potential use of these biomarkers in the detection of bovine chronic painful conditions.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The present study was financed by the Project CONICYT/FONDECYT/ INICIACION N° 11121615.

Animal Rights Statement: The Ethics and Bioethics Committee of the Universidad Austral de Chile approved the present study.

References

1. Allen K.A., Coetzee J.F., Edwards-Callaway L.N., Glyn H., Dockweiler J., KuKanich B., Lin H., Wang C., Fraccaro E., Jones M., Bergamasco L.: The effect of timing of oral meloxicam administration on physiological responses in calves

- after cauterizing dehorning with local anesthesia. *J Dairy Sci* 2013, 96, 5194–5205.
2. Banik R.K., Sato J., Giron R., Yajima H., Mizumura K.: Interactions of bradykinin and norepinephrine on rat cutaneous nociceptors in both normal and in lamed conditions *in vitro*. *Neurosci Res* 2004, 49, 421–425.
 3. Bäckryd E.: Pain in the blood? Envisioning mechanism-based diagnoses and biomarkers in clinical pain medicine. *Diagnostics* 2015, 17, 84–95.
 4. Bicalho R.C., Warnick L.D., Guard C.L.: Strategies to analyze milk losses caused by diseases with potential incidence throughout the lactation: a lameness example. *J Dairy Sci* 2008, 91, 2653–2661.
 5. Borsook D., Becerra L., Hargreaves R.: Biomarkers for chronic pain and analgesia. Part 1: the need, reality, challenges, and solutions. *Discov Med* 2011, 11, 197–207.
 6. Bustamante H.A., Rodríguez A.R., Herzberg D.E., Werner M.P.: Stress and pain response after oligofructose induced-lameness in dairy heifers. *J Vet Sci* 2015, 16, 405–411.
 7. Chen W., McRoberts J.A., Marvizón J.C.: M-opioid receptor inhibition of substance P release from primary afferents disappears in neuropathic pain but not inflammatory pain. *Neuroscience* 2014, 267, 67–82.
 8. Coetzee J.F.: A review of pain assessment techniques and pharmacological approaches to pain relief after bovine castration: Practical implications for cattle production within the United States. *Appl Anim Behav Sci* 2011, 135, 192–213.
 9. Coetzee J.F., Lubbers B.V., Toerber S.E., Gehring R., Thomson D.U., White B.J., Apley M.D.: Plasma concentrations of substance P and cortisol in beef calves after castration or simulated castration. *Am J Vet Res* 2008, 69, 751–762.
 10. Coetzee J.F., Mosher R.A., KuKanich B., Gehring R., Robert B., Reinbold J.B., White B.J.: Pharmacokinetics and effect of intravenous meloxicam in weaned Holstein calves following scoop dehorning without local anesthesia. *BMC Vet Res* 2012, doi: 10.1186/1746-6148-8-153.
 11. Corsi M.M., Fulgenzi A., Tiengo M., Pravettoni G., Gaja G., Ferrero M.E.: Effect of somatostatin on beta-endorphin release in rat experimental chronic inflammation. *Life Sci* 1999, 24, 2247–2254.
 12. Doehring A., Oertel B.G., Sittl R., Lötsch J.: Chronic opioid use is associated with increased DNA methylation correlating with increased clinical pain. *Pain* 2013, 154, 15–23.
 13. Galindo F., Broom D.M.: The relationships between social behavior of dairy cows and the occurrence of lameness in three herds. *Res Vet Sci* 2000, 69, 75–79.
 14. Hannibal K.E., Bishop M.D.: Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. *Phys Ther* 2014, 94, 1816–1825.
 15. Ji R., Berta T., Nedergaard M.: Glia and pain: is chronic pain a gliopathy? *Pain* 2013, 154, 10–28.
 16. Lay D.C. Jr., Friend T.H., Bowers C.L., Grissom K.K., Jenkins O.C.: A comparative physiological and behavioral study of freeze and hot-iron branding using dairy cows. *J Anim Sci* 1992, 70, 1121–1125.
 17. Lefcourt A.M., Elsasser T.H.: Adrenal responses of Angus x Hereford cattle to the stress of weaning. *J Anim Sci* 1995, 73, 2669–2676.
 18. Ley S.J., Livingston A., Waterman A.E.: Effects of clinically occurring chronic lameness in sheep on the concentrations of plasma noradrenaline and adrenaline. *Res Vet Sci* 1992, 53, 122–125.
 19. Ley S.J., Waterman A.E., Livingston A.: Measurement of mechanical thresholds, plasma cortisol, and catecholamines in control and lame cattle: a preliminary study. *Res Vet Sci* 1996, 61, 172–173.
 20. Lisowska B., Lisowski A., Siewruk K.: Substance P and chronic pain in patients with chronic inflammation of connective tissue. *PLoS ONE* 2015, 10, e0139206.
 21. Martini L., Whistler J.L.: The role of mu opioid receptor desensitization and endocytosis in morphine tolerance and dependence. *Curr Opin Neurobiol* 2007, 17, 556–564.
 22. O'Connor T.M., O'Connell J., O'Brien D.I., Goode T., Bredin C.P., Shanahan F.: The role of substance P in inflammatory disease. *J Cell Physiol* 2004, 201, 167–180.
 23. Osawa T., Nakao T., Moriyoshi M., Nakada K.: Plasma beta-endorphin around parturition and its relationship to cortisol level and resumption of pituitary and ovarian functions in dairy cows. *Anim Reprod Sci* 1998, 52, 27–38.
 24. Ossipov M.H., Morimura K., Porreca F.: Descending pain modulation and chronification of pain. *Curr Opin Support Palliat Care* 2014, 8, 143–151.
 25. Parent A.J., Beaudet N., Daigle K., Sabbagh R., Sansoucy Y., Marchand S., Sarret P., Goffaux P.: Relationship between blood- and cerebrospinal fluid-bound neurotransmitter concentrations and conditioned pain modulation in pain-free and chronic pain subject. *J Pain* 2015, 16, 436–444.
 26. Randall L.V., Green M.J., Chagunda M.G., Mason C., Green L.E., Huxley J.N.: Lameness in dairy heifer: impacts of hoof lesions present around first calving on future lameness milk yield and culling risk. *Prev Vet Med* 2016, 133, 52–63.
 27. Reader J.D., Green M.J., Kaler J., Mason S.A., Green L.E.: Effect of mobility score on milk yield and activity in dairy cattle. *J Dairy Sci* 2011, 94, 5045–5052.
 28. Saxler G., Löer F., Skumave M., Pfortner J., Hanesch U.: Localization of SP- and CGRP-immunopositive nerve fibers in the hip joint of patients with painful osteoarthritis and of patients with painless failed total hip arthroplasties. *Eur J Pain* 2007, 11, 67–74.
 29. Scholz J., Woolf C.J.: The neuropathic pain triad: neurons, immune cells, and glia. *Nat Neurosci* 2007, 10, 1361–1368.
 30. Shaw F.D., Tume R.K.: Beta-endorphin and cortisol concentrations in plasma of cattle. *Aust Vet J* 1990, 67, 423–424.
 31. Stewart M., Verkerk G.A., Stafford K.J., Schaefer A.L., Webster J.R.: Noninvasive assessment of autonomic activity for evaluation of pain in calves, using surgical castration as a model. *J Dairy Sci* 2010, 93, 3602–3609.
 32. Tsigos C., Reed P., Weinkove C., White A., Young R.J.: Plasma norepinephrine in sensory diabetic polyneuropathy. *Diabetes Care* 1993, 16, 722–727.
 33. Whay H.R., Waterman A.E., Webster A.J., O'Brien J.K.: The influence of lesion type on the duration of hyperalgesia associated with hindlimb lameness in dairy cattle. *Vet J* 1998, 156, 23–29.
 34. Whay H.R., Main D.C., Green L.E., Webster A.J.: Assessment of the welfare of dairy cattle using animal-based measurements: direct observations and investigation of farm records. *Vet Rec* 2003, 16, 197–202.