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Nociceptive threshold, blood constituents and physiological values in 213 cows with locomotion scores ranging from normal to severely lame

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ABSTRACT

The aim of this study was to investigate associations between mechanical nociceptive threshold, blood constituents, physiological measurements and locomotion score (LS) in dairy cattle with a range of LS from 1 (normal) to 5 (severely lame). The study used 213 Friesian/Friesian cross dairy cows from 12 farms. There were 40–50 cows each with LS 1–4 and 22 cows with LS 5. Each cow was restrained and her temperature and respiratory and cardiac rates were measured. Nociceptive threshold, plasma concentrations of haptoglobin, β-hydroxybutyrate (β-HB), cortisol, glucose, lactate, creatinine kinase activity, packed cell volume and white blood cell counts were determined. Mixed effect models were used to investigate associations between the variables measured and LS. Parity and stage of lactation were forced into all analyses and the model fit was checked by investigation of residuals.

After accounting for parity and stage of lactation, nociceptive threshold was significantly lower in cattle with LS 3–5 compared with LS 1 in a dose response manner, indicating increasing hyperalgesia with increasing LS. Haptoglobin concentration was raised in all cattle with LS > 1, demonstrating an inflammatory response with all levels of lameness. Cortisol and glucose concentrations were lower and β-HB concentrations higher in cows with LS 2 compared with cows with other scores, possibly signifying metabolic challenge. Heart and respiratory rate and rectal temperature were significantly higher only in cows with LS 5, suggesting that these measurements were insensitive measures of pain or stress. It was concluded that hyperalgesia increases with increasing severity of lameness and that nociceptive pressure and haptoglobin were sensitive measures of pain from lameness.

Introduction

Lameness is a serious animal welfare concern in dairy cows. It is painful (Whay et al., 1998; Winckler and Willen, 2001; Dyer et al., 2007) and causes economic losses (Esslemont and Kossaibati, 1997) through early culling (Booth et al., 2004) and reduced milk production (Amory et al., 2008; Green et al., 2010).

When in pain, many species of animal show behavioural indicators such as less curiosity towards the surrounding environment, vocalisation, reluctance to move and changes in facial expression (Molony and Kent, 1997; Underwood, 2002; Gregory, 2004). Lame cows have reduced daily activity (O'Callaghan et al., 2003), spend more time lying (Navarro, 2012) and less time eating (Galindo and Broom, 2002) and are more reluctant to interact with other cows. They are also more likely to be subjected to aggressive behaviours from healthy cows (Galindo and Broom, 2002). Finally, they also have physiological changes including alteration in heart rate, respiratory rate, temperature, blood pressure and pupil diameter (Molony and Kent, 1997; Lee, 2002). However, there is little research about metabolic changes in cattle during an episode of lameness (Belge et al., 2004; Calderon and Cook, 2011).

The nociceptive threshold test is used to measure a cow's response to a stimulus (Logue et al., 1998). Measurements of nociception have been used to investigate the effects of acute and chronic pain in cattle (Whay et al., 1997, 1998; Rushen et al., 1999), using heat radiation, thermal contact stimulation and mechanical stimulation (Ley et al., 1995; Whay et al., 1997, 1998; Herskin et al., 2003). The most relevant finding from these studies for the current work was that the threshold at which cattle responded to a mechanical stimulus was lower in lame cattle than in sound cattle (Ley et al., 1995; Whay et al., 1997), indicating a reduction in nociceptive threshold in cattle in chronic pain.

Raised levels of acute phase proteins (APPs) indicate inflammation. Healthy animals have low or undetectable baseline values with a narrow reference range which is unchanged with the age or sex of the animal (Kent, 1992). In ruminants, serum and plasma

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concentrations of haptoglobin increase during an acute phase response to episodes of infection, inflammation, immune disorders, trauma and stress (Eckersall and Bell, 2010; Kujala et al., 2010; Smith et al., 2010). Haptoglobin, a major APP, has been used as a physiological indicator of poor welfare (Arthington et al., 2003) and, more recently, raised levels have been associated with severe claw lesions in cattle (Smith et al., 2010; Kujala et al., 2010).

Pain caused by lameness could act as a stressor in dairy cattle (Underwood, 2002). Stress is associated with physiological variables such as body temperature, heart rate and respiratory rate (Caballero and Sumano, 1993; Tadich et al., 2000). Cortisol, despite its variability and short half-life, is still one of the most used indicators of stress (Cooper et al., 1995; Warriss et al., 1995; Ley et al., 1996; Tadich et al., 2005). Other blood constituents related to stress are packed cell volume, glucose, lactate, insulin, free fatty acids, creatinine kinase (CK) activity, catecholamines, β-endorphins, and β-hydroxybutyrate (β-HB) (Shaw and Tume, 1992; Cooper et al., 1995; Broom, 2003).

The aim of the current study was to examine the associations between mechanical nociceptive threshold, blood constituents, physiological values and locomotion in 213 dairy cattle with a range of locomotion scores from sound to severely lame.

Materials and methods

Twelve farms in the province of Valdivia, Chile, were accessed via their veterinarians. The farms were convenience selected, based on the willingness of the owner to participate, the distance between the farm and the university, appropriate roads to access the farms, easy access to the cows, facilities to examine feet and presence of lame cows. Farms were visited and cattle examined between May and December 2005; the number of visits per farm varied, depending on the number of lame cows on the farm at the time of the visit.

Two observers worked together (CT and SB). At each visit they identified non-lame cows and lame cows that were unilaterally lame on one hind limb and agreed on each cow's locomotion score (LS) (Sprecher et al., 1997) as they stood and walked on a concrete surface. Between 3 and 36 cattle were selected from each farm with a range of locomotion scores. In total 40–50 cattle with each of LS 1–4 and 22 cattle with LS 5 were selected for the study. Each cow was restrained in a metal crush where she was allowed to rest for 10 min. The heart rate and respiratory rate were then determined by thoracic auscultation. The rectal temperature was measured using a digital clinical thermometer (Digi-vet 35 T, Hauptner).

A mechanism to generate mechanical pressure for nociception was designed at the Faculty of Engineering at the Universidad Austral de Chile (Uribe et al., 2005). It consisted of an electronic servomotor with an acceleration sensor (Honeywell, model FSG15N1A), a spring and a blunt pin (2.5 mm diameter). The system was controlled by a programme written in Visual Basic for Windows via a laptop which translated and interpreted the commands given and performed the desired operation. The information was presented graphically and also stored in a spread sheet in MS Excel, interacting with the system through a serial port RS232 (Uribe et al., 2005).

The maximum force of the pin was 20 N to avoid injury to the animal. The device was placed 15 cm above the coronary band on the anterior surface of the metatarsus. In lame cows it was placed on the lame limb to avoid forcing the animal to bear weight on the affected limb, whilst for non-lame cows it was placed on the hind limb closest to the examiner (Whay et al., 1998). When activated, the device slowly and linearly increased pressure on the skin, measuring both force and acceleration. When the animal moved the limb in response to the pressure from the pin or when the pressure reached 20 N, the acceleration sensor automatically stopped and the maximum force recorded. The procedure was repeated four times on each cow with a 5 min rest between each measurement.

Finally, blood samples were collected from each animal by coccygeal venepuncture into evacuated tubes (Vacutainer, BD) with heparin and sodium fluoride (NaF). Plasma lactate (mmol/L) and glucose (mmol/L) concentrations were estimated from blood samples with NaF. Plasma cortisol (μg/dL), haptoglobin (mg/mL), CK (U/L), β-HB (mmol/L), packed cell volume (PCV) (%) and leukocyte concentration (1000/μL) were estimated from blood collected in heparin tubes. Blood samples were stored in insulated containers with ice packs at approximately 5 °C and transported to the laboratory immediately. At the laboratory they were centrifuged at 1330 *g* (Beckman TJ-6 centrifuge) at 24 °C for 10 min and the plasma stored at −20 °C until it was analysed.

Plasma cortisol concentration was measured by radioimmunoassay using commercial kits (Cortisol Coat-A-Count, DPC) validated for bovine plasma. The intra- and inter-assay coefficients of variation were 4% and 11%, respectively. The minimum detectable level of cortisol was 0.35 μg/dL. Glucose concentrations were determined using the GOD-PAP test without de-proteinisation (GL 2623, Randox). Lactate concentration was measured using the LOD enzymatic test. CK activity was measured using a UV-kinetic optimized method in an autoanalyser (Cobas Mira Plus, Roche). Haptoglobin was estimated in 103/217 cows with ~20 per LS (LS 1 = 19, LS 2 = 18, LS 3 = 26, LS 4 = 28 and LS 5 = 21) selected randomly using a commercial kit (Tridelta PHASE laboratory Haptoglobin Dev) and an autoanalyser (Cobas Mira Plus, Roche). The concentration of β-HB was estimated using 3-hydroxybutyrate dehydrogenase. The change from NAD+ to NADH was measured by spectrophotometer (Hitachi 4020 at 340 nm). PCV and leukocyte counts were done using a Sysmex KX-haematological counter.

The data obtained were stored in Microsoft Excel (Microsoft). Plots of the results were made in Minitab 16 and each outcome was analysed in a two level mixed effects model in MLwiN 2.25 (Rasbash et al., 2009) with penalised quasi-likelihood for parameter estimation that took the form:

$$Y_{ij} = \alpha + \beta X_{ij} + v_j + e_{ij}$$

where $v_j \sim N(0, a_v^2)$ and $e_{ij} \sim N(0, a_e^2)$

Y_{ij} is the mean nociceptive threshold value, blood constituent values and physiological values in turn where the subscripts *i*, and *j* denote the *i*th cow on the *j*th farm, α the regression intercept, X_{ij} the vector of covariates associated with each cow, β the coefficients for covariates X_{ij}, v_j a random effect to reflect residual variation between farms and e_{ij} residual variation between cows, both with mean zero and variance a^2.

Locomotion score, parity (categorised 1, 2 ... 5, 6+) and stage of lactation (a categorical variable early, mid, late, dry) were forced into each model. A difference of $P < 0.05$ was considered statistically significant. The model fits were checked. Correlations between the outcome variables were investigated.

This study was approved by the University Ethical Committee and the Chilean Government Committee for the Use of Animals for Research. After the observations were made, lame cows were treated by the farm hoof trimmer appropriately for the cause of lameness. The observation, recording of LS and blood sampling of the cows did not produce any delay in the treatment of lameness.

Results

All results from models were adjusted for parity and stage of lactation. There was no significant residual variation between farms, indicating that there was no large farm effect in the data but there was significant residual variation between cattle (data not shown).

The mean nociceptive threshold was 13.63 N (SE 1.0) in cows with LS 1. It decreased in a dose response manner as LS increased (Table 1). Cows with LS 3, 4 and 5 had significantly lower nociceptive thresholds than cows with LS 1 (Table 1); cows with LS 4 and LS 5 had significantly lower thresholds than cows with LS 2 (checked by altering the baseline for comparison). Cows with LS 1 had a mean haptoglobin of 0.06 mg/mL, the baseline category of non-lame cows, which was within the reference range (as expected in non-lame cows). All cattle with LS > 1 had haptoglobin concentrations that were significantly above cows with LS 1 and above the reference range of <0.1 mg/mL, indicating that haptoglobin was a sensitive marker for all levels of lameness. There was a negative correlation between mean nociceptive pressure and haptoglobin ($r = -0.16$; $P < 0.05$) indicating that haptoglobin was positively correlated with allodynia.

Respiratory rate was slightly above the reference range for all animals; heart rate and rectal temperature were in the reference range for all cattle except those with LS 5. Cattle with LS 5 had significantly higher mean rectal temperature, heart rate, and respiratory rate than those with LS 1; cattle with LS 2–4 did not have values significantly different from non-lame ones (LS 1) indicating that rectal temperature, heart rate, and respiratory rate were not sensitive measures of pain except in severely lame cows (Table 1). These three variables were positively correlated with haptoglobin levels ($r = 0.23$, $r = 0.14$, $r = 0.12$ respectively; $P < 0.05$). In addition, heart rate was positively correlated with rectal temperature ($r = 0.25$; $P < 0.001$), respiratory rate ($r = 0.31$; $P < 0.001$) and β-HB ($r = 0.21$; $P < 0.01$); heart rate was positively correlated with β-HB and rectal temperature was negatively correlated with nociceptive pressure ($r = -0.14$; $P < 0.05$); rectal temperature and respiratory rate were positively correlated with leukocyte count ($r = 0.27$; $P < 0.001$ and $r = 0.22$; $P < 0.01$) respectively.