© 2010 Universities Federation for Animal Welfare
The Old School, Brewhouse Hill, Wheathampstead,
Hertfordshire AL4 8AN, UK

Animal Welfare 2010, 19: 31-36
ISSN 0962-7286

The use of a hand-held algometer as a method to measure mechanical nociceptive thresholds in sheep

SM Stubsjøen*†‡, PS Valle†§ and AJ Zanella†

† Norwegian School of Veterinary Science, Department of Production Animal Clinical Sciences, PO Box 8146, Dep NO-0033 Oslo, Norway
‡ Animalia, PO Box 396, Økern, NO-0513 Oslo, Norway
§ Molde University College, PO Box 2110, NO-6402 Molde, Norway
* Contact for correspondence and requests for reprints: solveigmarie.stubsjoen@nvh.no

Abstract

*The aim of this study was to explore the use of a hand-held algometer for the measurement of mechanical nociceptive thresholds (MNT) in sheep (*Ovis aries*). Twelve ewes were tested over three consecutive days by two operators, and MNTs were measured over six predetermined sites on both forelimbs every five minutes for 30 min. The effects of test period, measurement number within test period and different anatomical points on MNT levels were investigated, in addition to establishing baseline MNT levels for the sheeps' forelimbs. A significant decrease of MNT values was observed over the three consecutive test days and within each test period. The anatomical points located closest to the carpus and fetlock joints had significantly higher MNT values compared to the anatomical points located over the middle part of the metacarpus, possibly due to the protective function of the distal part of the extensor retinaculum and the dorsal pouch of the fetlock joint capsules. There was no difference in MNT values between the right and left foreleg. There was a tendency for a flattening out of the drop in MNT towards the last measurement. Hence, we suggest using the values from the last two measurements when determining normative values, and to habituate the ewes to the procedure of measuring MNT levels. Taking these factors into consideration, a hand-held algometer is a useful tool to measure MNTs in sheep.*

Keywords: *algometer, animal welfare, mechanical nociceptive threshold, metacarpus, pain, sheep*

Introduction

The ability to quantitatively assess pain is an important component in the improvement of animal welfare. A better understanding of pain experienced by sheep is necessary in order to address welfare issues related to sheep production. Pressure algometry provides a quantitative assessment of mechanical pain by the application of blunt pressure on a section of skin/muscle (Fischer *et al* 1987). The mechanical nociceptive threshold (MNT) is defined as the amount of applied pressure necessary to produce a behavioural response indicative of pain (Haussler *et al* 2007). MNTs may be used to assess the effectiveness of anaesthetic and analgesic interventions; to monitor the effect of treatment on chronic pain conditions; to map the degree of wound hyperalgesia after surgery and to assess the nervous system changes associated with clinical or experimental procedures (Slingsby *et al* 2001).

Different devices to measure MNTs have been developed for use in large animals (Nolan *et al* 1987; Chambers *et al* 1994). A hand-held algometer has the added advantage that MNT testing may be performed on multiple areas of the body. If an algometer is to be used to assess the effect of an intervention it must first be shown to be reliable (Potter *et al* 2006). Thus, the objective of this study was to investigate: (i) baseline MNTs over six points in the sheeps' foreleg and (ii) the effects of test period, measurement number within test period and different anatomical points measured over the metacarpus, on MNT levels.

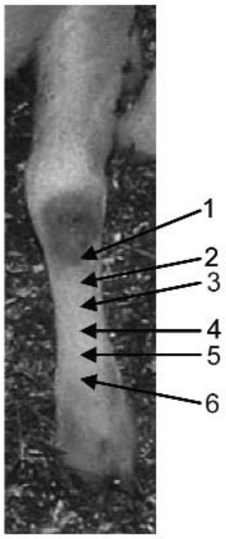
Materials and methods

Twelve, non-lactating, non-pregnant, 2- to 4-year old Norwegian White ewes with a mean bodyweight of 80 kg (range: 71 to 105 kg) were used. The ewes were selected using a simple random sampling technique from a flock of 260 ewes in Sæter Experimental Farm in Norway. A clinical examination of the selected ewes was performed, and existing health records were examined to exclude any diseased or injured animals prior to the experiment. The ewes were fed hay and concentrate twice daily and had *ad libitum* access to water.

Each ewe was tested in an experimental pen while remaining in visual contact with other ewes. The electronic algometer used (Commander Algometer, JTECH Medical Industries, Salt Lake City, Utah, USA) had a blunt plastic tip of 0.5 cm^2. The algometer was held perpendi-

https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press

Figure 1



Mechanical nociceptive threshold (MNT) values were measured over six points located on the midline of the cranial aspect of the metacarpus on both forelimbs.

Table 1 Multilevel model to show the effects of test period, measurement number, anatomical points on leg and the individual ewe on mechanical nociceptive threshold (MNT) levels in Newton (N).

Parameter	Coefficient estimate	SE	*P*-value
	Fixed		
Constant/Intercept	36.40	1.64	< 0.001
Test period	–5.15	0.60	< 0.001
Measurement number	–0.77	0.19	< 0.001
Points 1 and 6 on leg	4.38	0.68	< 0.001
	Random		
Sheep	5.00	3.27	0.13
Test period	10.84	3.21	< 0.001
Measurement number	39.15	2.92	< 0.001

cular to predetermined sites on the forelimb (see Figure 1), and the rate of force application was approximately 2 Newton sec^{-1} 0.5 cm^{-2}. The MNTs over six points located on the midline of the cranial aspect of the metacarpus on both forelimbs were measured in a randomised order every five minutes for 30 min. The cut-off point of the force applied to the animal's leg was 50 Newton to avoid tissue damage. Avoidance reaction was defined as lifting the forelimb off the ground or a clear shifting of weight. The operator then released the pressure and removed the algometer. Each ewe was tested during three consecutive days, and the order of the ewes was randomised for each test period. The two operators conducting the study followed precise time guidelines when applying pressure to the sheep's foreleg, as the rate of pressure increase may be a factor affecting reliability of the responses.

To impose pain on animals deliberately raises ethical concerns (Nolan *et al* 1987). The hand-held algometer allowed differentiation between innocuous and noxious stimuli without producing visible tissue damage. The leg-withdrawal response is a natural response of the animal, and there was an easily-defined endpoint, which lead to a rapid termination of the stimulus.

The protocol to conduct this study was approved by the National Animal Research Authority in Norway.

Statistical analysis

Descriptive analysis was performed in Excel® and SAS®, and the data were further analysed and tested for significant effects ($P < 0.05$) using MLwiN® (Rashbash *et al* 2005), which handles the structure with repeated measurements (six measurements on different points on each of the two forelegs, over three periods on the same ewe [n = 12]). Sheep were kept (independent of *P*-value) as a random effect in the model, while the other levels in the repeated/random structure (measurement number, leg and test period) were tested for significant effects ($P < 0.05$). Linear relationships between explanatory variables (eg test period) and the outcome was chosen based on graphical examinations, and in order to reach a parsimonious model.

Results

Graphical evaluation indicated a decrease in MNT values over the test periods (Table 1, Figures 2 and 3) as well as over the six consecutive measurements (Figure 4) carried out in each leg. We also observed a difference in value for the anatomical points on the leg (Figure 5) with point 1 and 6 (Figure 1) showing higher MNT values than the points from 2 to 5 ($P < 0.001$).

Based on the multilevel model results (Table 1) we found a lowering ($P < 0.001$) of MNT values over the three test periods. The MNT values also decreased in a linear manner over the number of measurements within each test period ($P < 0.001$). The tendency for a flattening out of MNTs towards the final measurement, indicated by the graphical investigations (Figure 4), was not statistically confirmed. No difference in MNT values was found between the right and left foreleg, and age and weight showed no significant effect in the model.

There was a considerable, but non-significant, variability in MNT values across subjects.

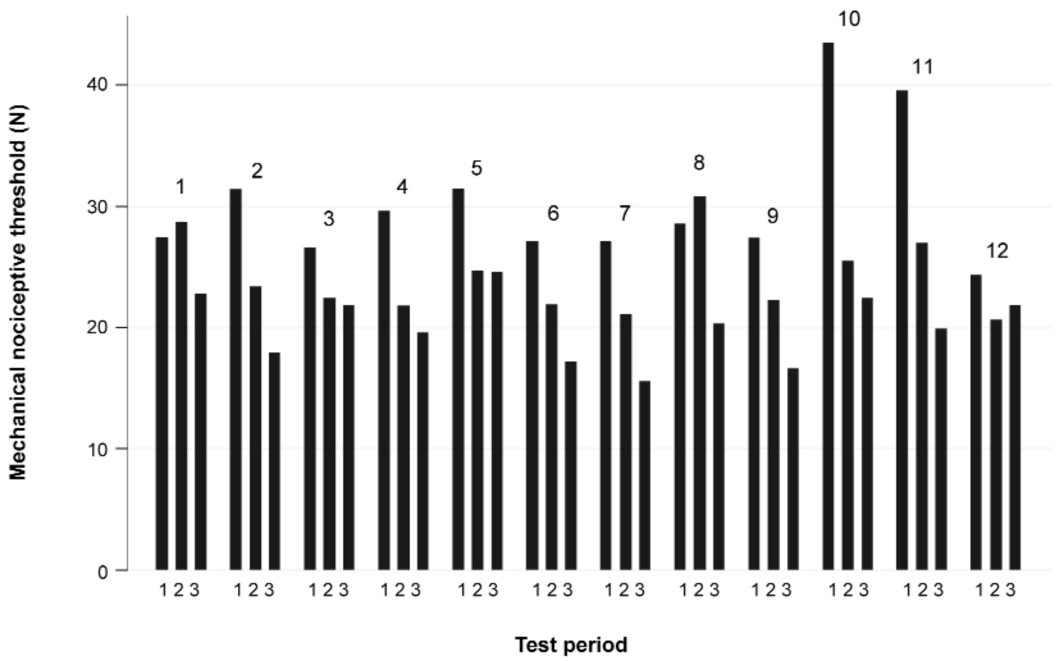
Altogether, there was not a strong reproducibility for the MNT measurements, which vary especially according to where in the sequence of repeated measurements (ie test period and/or measurement number) the measure is taken.

Discussion

In our study, we found a decrease in MNT values observed over the three consecutive test days, which is in accordance with a study performed in humans by Jones and colleagues (2007). We propose that this finding, in addition to the decrease in MNTs observed over six consecutive measure-

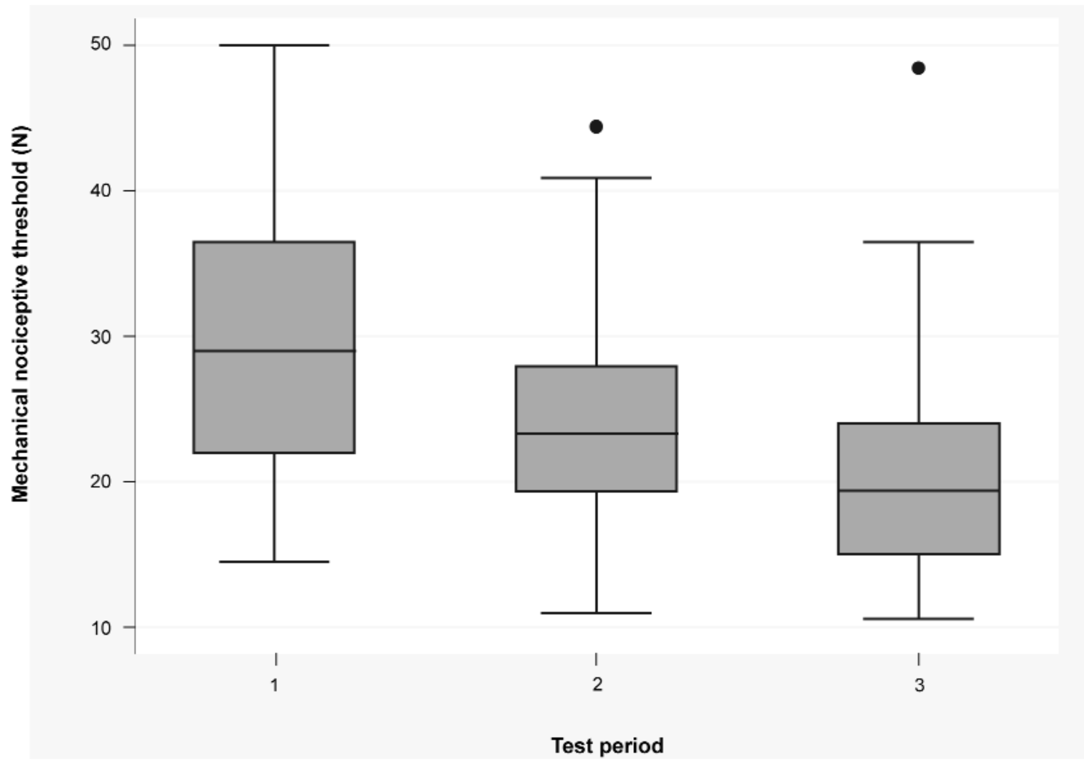
https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press

Figure 2



Mean mechanical nociceptive threshold (MNT) values in Newton (N) of 12 ewes measured over three consecutive test days. Data are presented as mean value of six measures obtained in each front leg of the tested animals.

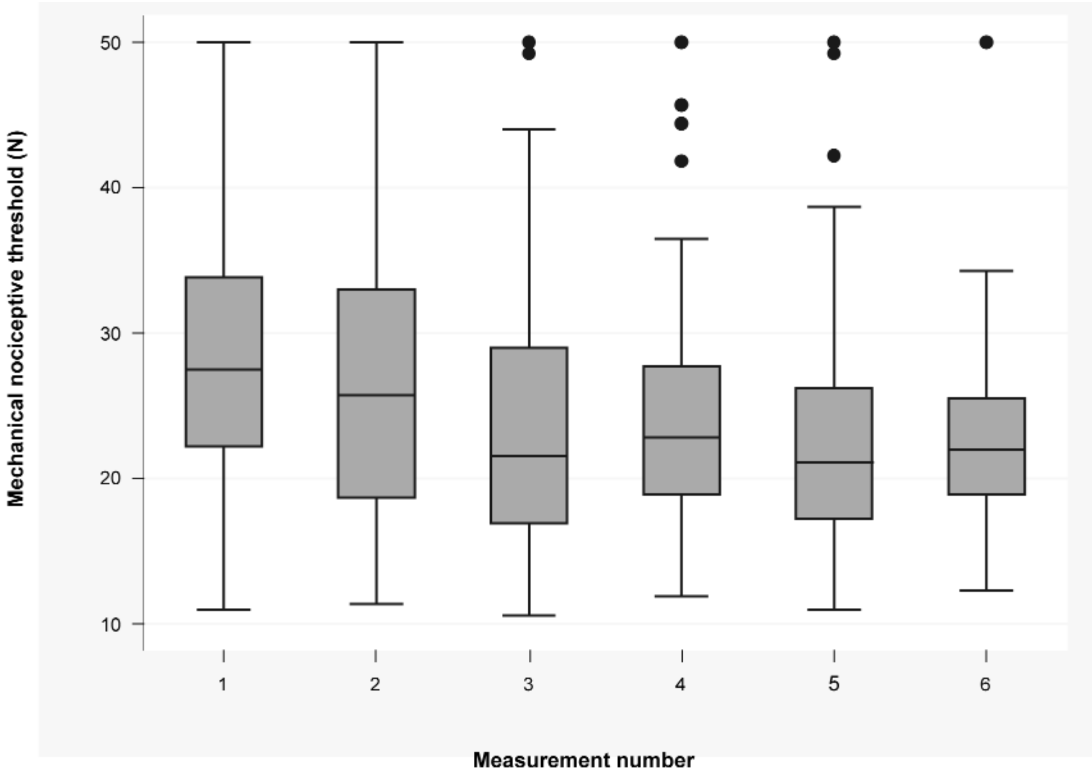
Figure 3



Mean and 95% confidence interval of mechanical nociceptive threshold (MNT) values in Newton (N) measured over three consecutive test days in 12 ewes. Data are presented summarising all the measured values for the tested animals.

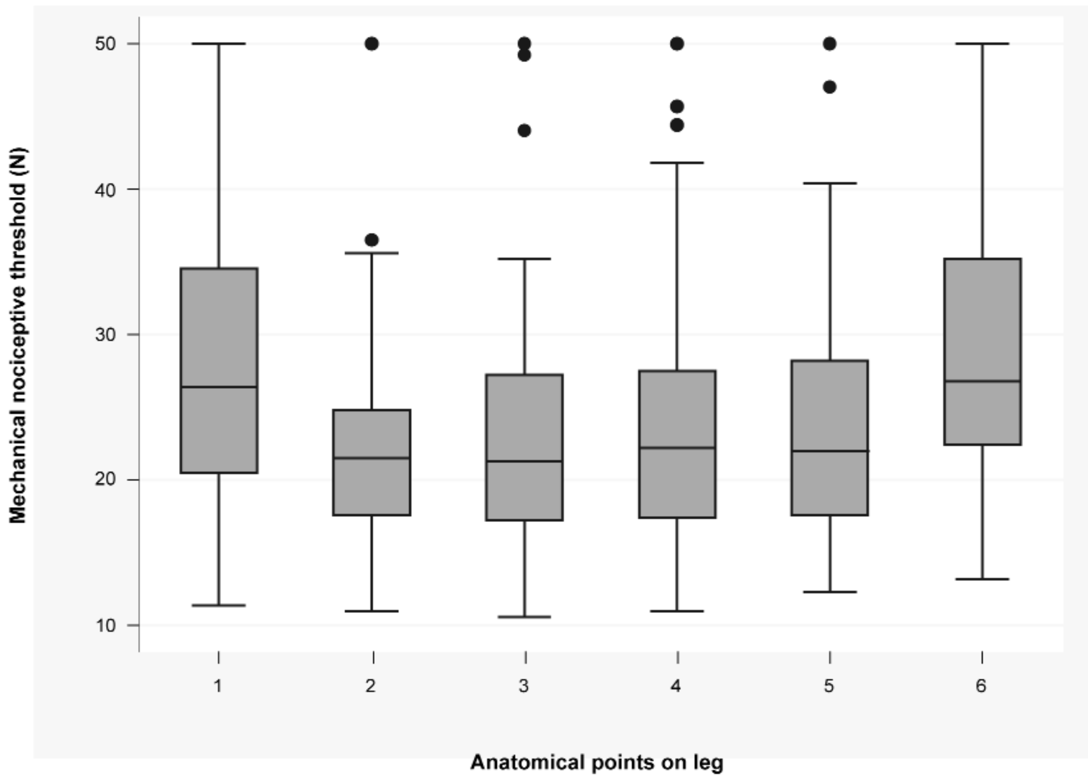
https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press

Figure 4



Mean and 95% confidence interval of mechanical nociceptive threshold (MNT) values in Newton (N) of 12 ewes for measurement number 1–6 obtained over three consecutive test days.

Figure 5



Mean and 95% confidence interval of mechanical nociceptive threshold (MNT) values in Newton (N) of 12 ewes measured over points 1–6 (see Figure 1) during three consecutive test days.

https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press

ments, might be due to some form of learned behavioural response (Jones *et al* 2007). Mechanical nociceptive values can be affected by adaptation of the subject to the procedure (List *et al* 1991). A short sound was released from the algometer at the moment when the force application reached 4 N, and this may have contributed to adaptation and hence reduced the MNT values. However, we speculate that the sound may have had a positive effect in promoting a shift in the attention focus of the animals. The sound coming from the algometer could have possibly altered the ewes' motivational state, making them aware that the procedure of measuring MNTs was starting. This could be an advantage, because the ewes would be likely to be in a comparable motivational state when the MNTs were obtained, which again would produce a specific behaviour (leg-withdrawal response) and give more accurate results.

We consider it highly unlikely that increased sensitisation of localised nerve receptors and local tissue trauma may have caused reduced MNT values over consecutive days as suggested by Jones and colleagues (2007). In this experiment, we did not notice any signs of local inflammation or lameness in the experimental animals.

We cannot discard the possibility that some form of central response is involved in the lowering of MNT values. It is unclear whether the repeated response to MNT testing over consecutive days was sufficient stimulation to cause some form of physiological adaptation.

In our study, we found a considerable, but non-significant, variability of MNTs across the ewes studied. Other investigators have found large variability across subjects when determining normative values in humans (Jensen *et al* 1986; Rolke *et al* 2005), supporting our decision to keep the ewe factor in the random part of the model even though not significant at the $P < 0.05$ level. The likely explanation for this finding is the low number of ewes studied and within-subject variability observed across test periods.

Mean pain pressure thresholds in healthy sheep have been found to be lower in a previous study (4.87 [± 0.078] N; Chambers *et al* 1994) compared to our study. We used a blunt plastic tip of 0.5 cm^2, in contrast to a 2-mm diameter steel pin reported in the study by Chambers *et al* (1994). When applying the hand-held algometer, the operator was in close proximity to the animal. Chambers and colleagues used a device which was controlled by a computer and started by a foot switch and the animals were possibly less disturbed during the procedure.

In different species, freezing behaviour as part of the reactive coping response can be observed in response to an inescapable stressor or predator (Koolhaas *et al* 1999). The mean MNT values measured in ewe numbers 10 and 11 during test period 1 (see Figure 2) were high, almost reaching the established threshold. We speculate that these MNT values were caused by a fear-induced behavioural inhibition, and could hence have contributed to the variability of MNTs over test periods and across ewes. Interestingly, there was no influence of animals' age and weight on the MNT values.

The variation in MNTs was not found to be related to the two operators conducting the study. However, inter-observer reliability needs to be assessed in a study with several operators.

Haussler and colleagues (2007) found reduced variability in MNT values at proximal sites within the proximal portion of the thoracic limb, compared with distal sites in horses. More consistent avoidance reactions were observed within the axial skeleton compared to the appendicular skeleton. Withdrawal or avoidance reactions of the thoracic limbs were thought to be more difficult because of the effects of weight-bearing. This may also be true for ewes, making greater variability in MNT values when these are being measured over the metacarpus. No difference in MNT values was found between the right and left foreleg.

The points 1 and 6 on the forelegs had significantly higher MNT values compared to points 2–5 (Figure 1) in our study. Pain pressure thresholds have been found to depend on tissue type, and to be higher over both nail bed and bony prominences than over muscle in humans (Rolke *et al* 2005). Points 1 and 6 were located near to the carpus and fetlock joints, respectively. The fibrous layer of the joint capsule covering the carpal bones blends dorsally with the thick, deep fascia (extensor retinaculum) that holds the extensor tendons in place. The capsule of the fetlock joint extends proximally as a dorsal pouch between the metacarpal bone and the extensor tendons (Dyce *et al* 1996). The higher pain threshold for points 1 and 6 compared to points 2–5 might be due to the protective function of the distal part of the extensor retinaculum and the dorsal pouch of the fetlock joint capsules. Application of pressure over the middle part of metacarpus would be expected to activate the periosteum, which has marked pain sensitivity (Grönblad *et al* 1984).

This study is the first step in the validation of a hand-held algometer for measuring MNTs in sheep. Further research is needed to assess MNTs in sheep experiencing pain. Animals in pain would be expected to have lower MNTs than healthy animals, as previously found in sheep with footrot (Chambers *et al* 1994).

Animal welfare implications

In our study, we offered an objective protocol to assess MNTs in sheep. We expect that our contribution will be valuable in the much-needed work to develop reliable, quantifiable methods to assess pain in sheep, thereby improving their welfare.

Conclusion

Since there is a tendency for a flattening out of the drop in MNTs towards the final measurement, we suggest that the values from the last two measurements should be used when determining normative values. The ewes should be habituated to the procedure of measuring MNT levels in order to reduce the variability. When taking these factors into consideration, we suggest that a hand-held algometer is a useful tool to measure MNTs in sheep.

https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press

Acknowledgements

The authors wish to acknowledge the assistance of Mr Ronald Stai at Sæter Experimental Farm, Bioforsk, Norway. The Norwegian Research Council, NORTURA and 'Fondet for forskningsavgift på landbruksprodukter' are acknowledged for funding this study.

References

Chambers JP, Waterman AE and Livingston A 1994 Further development of equipment to measure nociceptive thresholds in large animals. *Journal of Veterinary Anaesthesia 21*: 66-72

Dyce KM, Sack WO and Wensing CJG 1996 *Textbook of Veterinary Anatomy. The Forelimb of the Ruminants* pp 737-757. WB Saunders: Philadelphia, USA

Fischer AA 1987 Pressure algometry over normal muscles: standard values, validity and reproducibility of pressure threshold. *Pain 30*: 115–126

Grönblad M, Liesi P, Korkala O, Karaharju E and Polak J 1984 Innervation of human bone periosteum by peptidergic nerves. *The Anatomical Record 209*: 297-299

Haussler KK, Hill AE, Frisbie DD and McIlwraith CW 2007 Determination and use of mechanical nociceptive thresholds of the thoracic limb to assess pain associated with induced osteoarthritis of the middle carpal joint in horses. *American Journal of Veterinary Research 68*: 1167-1176

Jensen K, Andersen HO, Olesen J and Lindblom U 1986 Pressure-pain threshold in human temporal region. Evaluation of a new algometer. *Pain 25*: 313-323

Jones DH, Kilgour RD and Comtois AS 2007 Test-retest reliability of pressure pain threshold measurements of the upper limb and torso in young healthy women. *The Journal of Pain 8*: 650-656

Koolhaas JM, Korte SM, de Boer SF, Van der Vegt BJ, Van Reenen CG, Hopster H, De Jong IC, Ruis MAW and Blokhuis HJ 1999 Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience & Biobehavioral Reviews 23*: 925-935

List T, Helkimo M and Karlsson R 1991 Influence of pressure rates on the reliability of a pressure threshold meter. *Journal of Craniomandibular Disorders 5*: 173-178

Nolan A, Livingston A, Morris R and Waterman A 1987 Techniques for comparison of thermal and mechanical nociceptive stimuli in the sheep. *Journal of Pharmacological Methods 17*: 39-49

Potter L, McCarthy C and Oldham J 2006 Algometer reliability in measuring pain pressure threshold over normal spinal muscles to allow quantification of anti-nociceptive treatment effects. *International Journal of Osteopathic Medicine 9*: 113-119

Rasbash J, Steele F, Browne WJ and Prosser P 2005 *A User`s Guide to MLwiN Version 2.0. Centre for Multilevel Modelling.* University of Bristol: Bristol, UK

Rolke R, Andrews Campbell K, Magerl W and Treede R-D 2005 Deep pain thresholds in the distal limbs of healthy human subjects. *European Journal of Pain 9*: 39-48

Slingsby LS, Jones A and Waterman-Pearson AE 2001 Use of a new finger-mounted device to compare mechanical nociceptive thresholds in cats given pethidine or no medication after castration. *Research in Veterinary Science 70*: 243-246

© *2010 Universities Federation for Animal Welfare*

https://doi.org/10.1017/S0962728600001135 Published online by Cambridge University Press