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Dehorning and welfare indicators in beef cattle – A meta-analysis

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Welfare and dehorning in cattle: a meta-analysis

Abstract. Dehorning is a common practice in cattle farming. Researchers suggest that pain during dehorning can be mitigated, although there is no conclusive evidence about the best technique and the best manner of pain relief. A systematic review-meta-analysis was performed to clarify the effect of dehorning on welfare indicators (cortisol concentration or average daily gain [ADG] or vocalisation) in beef cattle up to 12 months of age. Five electronic databases were systematically searched, as well as conference proceedings and experts were contacted electronically. Pre-defined protocols were applied during all steps of the systematic review process. A random effect meta-analysis was conducted for each indicator separately with the mean of the control and treated groups. Four publications reporting 7 studies and 69 trials were included in the MA involving 287 cattle. Heterogeneity between studies was observed for cortisol (I^2 = 50.5%), ADG (I^2 = 70.5%), and vocalisation (I^2 = 91.9%). When comparing the non-dehorned group with amputation dehorning, the cortisol concentration was lower 30 min (P < 0.0001) and 120 min (P = 0.023) after procedure (0.767 nmol/L and 0.680 nmol/L, respectively). Local anaesthesia did not show a reduction in cortisol concentration at 30 min after dehorning by amputation. Non-dehorned animals had a tendency to decrease the number of vocalisation (P = 0.081; *MD* = 0.929) compared with the group dehorned by amputation. These results suggest that dehorning is a painful experience and that local anaesthesia did not alleviate short-term pain following dehorning. Further investigation into pain relief is required to improve confident decision making under practical conditions.

Additional keywords: animal analgesics, animal pain, animal welfare, cattle

Introduction

The prevention of horn growth (disbudding) or removal of horns (dehorning) are commonly performed practices in the beef cattle industry (Stafford and Mellor 2005). Regardless of the technique, disbudding and dehorning generate a pain-induced response, which can be alleviated by applying strategies to alter the threshold of pain or decrease the transmission of impulse in pain nerves from the wound (Sylvester *et al.* 1998*b*). Despite the evidence, the procedures are often performed without administering analgesics (Stewart *et al.* 2009; Theurer *et al.* 2012). The recognition and assessment of pain following painful procedures through a combination of physiological, behavioural and production responses have been recommended (Stafford and Mellor 2005).

Management practices have been adopted to dehorn cattle for better farm management (Stock *et al.* 2013). Hornless cattle reduce the risk of injuries to humans and other animals in the herd, require less feeding-trough space and decrease the incidence of carcass wastage due to bruising (Faulkner and Weary 2000; Stafford and Mellor 2005; Stock *et al.* 2013). However, the well-being of cattle undergoing dehorning has been of great public concern.

The literature focusing on pain management in cattle during dehorning and disbudding is plentiful (McMeekan *et al.* 1998; Schwartzkopf-Genswein *et al.* 2005; Doherty *et al.* 2007; Sinclair, 2012; Hubber *et al.* 2013). The current state of knowledge about these procedures and their relationship with pain alleviation have been discussed subjectively in traditional reviews (Stafford and Mellor 2005, 2011). However, it is crucial clarify the technique which causes the least pain and the best pain relief to minimize pain-induced distress (Stafford and Mellor 2011; Vickers *et al.* 2005; Theurer *et al.* 2012). Hence, due the variability and difficulties in field research, the systematic review (SR) and meta-analysis (MA), by integrating the findings from many studies, can synthesize and increase the credibility of the results, providing a more robust estimate of effect (Egger *et al.* 2001; Borenstein *et al.* 2009).

A rigorously conducted MA could provide new insights into animal well-being (Lean *et al.* 2010; Canozzi *et al.* 2017). We conducted a SR-MA to test the hypothesis that strategies, i.e. specific techniques and/or pain relievers, could be used to prevent or minimize the negative impacts of dehorning/disbudding on beef cattle. The goal of this study was to summarize all available scientific evidence on the effects of both procedures, and the efficacy of pain relief on beef cattle welfare using a SR-MA approach.

Material and methods

Data source and searches

Studies were systematically identified by searching electronic databases and grey literature sources (conference proceedings, theses and government or research station reports). The internet servers of the Federal University of Rio Grande do Sul (UFRGS, Brazil) and of the National Research Institute for Agriculture (INIA, Uruguay) were used to cover CAB Abstracts (Thomson Reuters, 1910–2015), ISI Web of Science (Thomson Reuters, 1900–2015), PubMed (1940–2015), Agricola (EBSCO, 1970–2015) and Scopus (Elsevier, 1960–2015) up to May 2015. Additionally, the main conferences in animal production and ethology - Joint Annual Meeting, JAM (from 2001 to 2014) and International Society for Applied Ethology, ISAE (from 2001 to 2014), respectively - had their proceedings scanned for references. Efforts were made to use unpublished data and animal welfare researchers were contacted by electronic mail. In addition, we screened the bibliographies of published literature reviews for potential eligible reports (Stafford and Mellor 2005; Weary *et al.* 2006; Stafford and Mellor 2011; Schwartzkopf-Genswein *et al.* 2012).

The review question was defined based on key concepts in terms of PICO: population (P), intervention (I), comparator (C), and outcome (O). The studied population was beef cattle up to 12 months of age (calf and/or yearling), since the experience of intense pain soon after birth may “programme” the animal’s subsequent sensitivity to pain challenges (Viñuela-Fernández et al., 2007). The present study only shows findings on dehorning and disbudding interventions; however, the literature search was conducted to also include castration, as presented in Fig. 1. The comparison groups considered were similar groups of cattle undergoing the same procedure, with or without intervention. We did not exclude studies based on the type of comparison used. Vocalisation, cortisol, and average daily gain (ADG) were the interest outcomes.

(Insert Fig. 1 here)

(Insert Table 1 here)

The literature search strategy comprised the following key words: (bovine OR "beef cattle" OR cal* OR herd) AND (disbud* OR dehorn* OR castration) AND (“animal wel*" OR "animal pain" OR "animal stress" OR cortisol OR behavio* OR vocali*). This search strategy also retrieved studies, which measured animal performance. Therefore, “average daily gain” was not included to avoid an overload of non-relevant citations.

All references were downloaded into the reference manager RefWorks (RefWorks–COS, USA) and duplicates were removed manually.

Selection of papers

Studies were included or excluded in this SR based on a standardized form, which was adapted from previously published protocol (Mederos *et al.* 2012). Five reviewers, who were trained for the relevance screening step using 30 abstracts, audited the review process.

Titles and abstracts (when available) of publications identified by the searches were independently assessed for potential inclusion by two members. Discrepancies were discussed and disagreements resolved through consensus or referral to a third reviewer.

Inclusion and exclusion criteria. The candidate studies were included if the study resulted in full manuscript from peer-reviewed journals; evaluated the animal welfare in beef cattle; investigated castration or dehorning or disbudding; and analysed cortisol level, vocalisation or ADG as welfare indicators.

The study designs included randomized and non-randomized clinical trials, cohort studies, and case-controls. In order to maximise sensitivity we did not restrict language or publication year.

An electronic SRSnexus review format (V. 5.0, Möbius Analytics, Ottawa, Ontario, Canada) was used for all SR steps.

Data extraction strategy and manipulation

Data extraction (DE) forms were adapted from previous studies and were completed by the first author. If the publications reported more than one study design, data for each study were recorded separately.

Before risk of bias assessment and DE, the relevance of papers selected through abstract screening was confirmed using the full papers based on language (English, Spanish, Portuguese, or Italian); appropriate control group; sufficiently detailed to conduct the DE and to extract quantitative data to perform the MA. At this stage, primary research was restricted to publications in those languages that the research team members were fluent, since the translation was precluded due to financial constraints.

Study details included population, intervention, outcome measurements, results, and manuscript information (journal name, author(s) name(s), year of publication, and original language). For the purpose of clarity, throughout this manuscript both procedures, i.e. dehorning or disbudding, will be used as in the original manuscript. For each outcome, we attempted to assemble the following information: mean, standard deviation (SD) or any available measure of dispersion, measurement unit, *P*-value, and the number of animals in the control and treatment groups. All results from cortisol were transformed to nmol/L and from ADG to g/day.

An Excel sheet was built with the extracted data, as well as dataset containing the results for controlled trials, measuring cortisol (baseline, 20 or 30 or 40 min, and 120 min), ADG (during observation period) or number of vocalisations (during intervention). Moreover, the research team stratified the methods into three groups: 1) amputation using scoop dehorners, such as Barnes, Keystone, knife, and cup (plus cautery iron);

2) cauterization using hot iron (electric or thermal); and 3) amputation *vs.* cautery dehorning.

The control group could have been non-dehorned (Group 1 and 2) or subjected to amputation (Group 1) or cautery (Group 2) dehorning, and the treated group was always submitted to amputation (Group 1) or cautery (Group 2) dehorning. When the comparison was between two dehorned groups, the intention was to compare different techniques of amputation (Group 1) or cautery (Group 2) dehorning. In addition, relevant pain relief strategies were stratified as anaesthesia (lidocaine, procaine, and Tri-Solfen®), non-steroidal anti-inflammatory drug (NSAID; meloxicam), and multimodal therapy (combination of flunixin and procaine, and lidocaine and meloxicam).

When the results were reported in the log-transformed scales, these were transformed back to the original scale using the formula described by Mederos *et al.* (2012). A pooled standard deviation (Sp) was based on the formula when an overall standard error of the mean (SEMp) was mentioned for the control and treatment groups (Ceballos *et al.* 2009; Higgins and Green 2011; Mederos *et al.* 2012):

$$S_p = SEM_p \times \sqrt{n_p}$$

Where Sp is the pooled standard deviation and n_p is the number of calves in the treatment and control groups.

Studies that reported only *P*-value, an estimation of a common SD was obtained using the *t*-statistic under the assumption that the data was normally distributed (Ceballos *et al.* 2009; Mederos *et al.* 2012):

$$S_P = \frac{(x_2 - x_1)}{t(\alpha dfE)\sqrt{(1/n_2) + (1/n_1)}}$$

Where $x_2 - x_1$ represents the means difference; $t(\alpha f dE)$ is the percentile from the reference distribution; and n is the sample size of each group.

Additional considerations in the data-extraction step were as follows: when results were presented as graphics, the corresponding author was contacted by electronic mail and asked to provide the summary statistics. If no response was obtained or data were not provided, the mean and/or measure of dispersion were manually extracted using a ruler. Since the cortisol data were collected in three different times, the summary data were recreated and the effect size was computed according to recommended approaches (Borenstein *et al.* 2009).

Assessment of risk of bias

The form to assess the risk of bias was based on questions suggested in the Cochrane Handbook (Higgins and Green 2011), with one minor modification. The domain "blinding of outcome assessment" was considered at high risk of bias if blinding was not reported and at low risk if blinding was reported for vocalisation (Dzikamunhenga *et al.,* 2014), since it is a subjective measure and more prone to poor reliability (Weary *et al.* 2006). Otherwise, regardless of the presence or absence of blinding, cortisol and ADG were considered to be at low risk of bias. All outcomes were evaluated by domain and the first author performed assessment.

Statistical analysis

The Stata statistical package (version 14, StataCorp., College Station, TX, USA) was used to analyse each outcome by mean difference (MD) between control and treatment groups with a 95% confidence interval (95% CI). Data analysed for cortisol were obtained from baseline to 20/30/40 min and up to 120 min; for ADG, during the follow-up period reported by the authors; and for vocalisation, during the dehorning or disbudding. For cortisol, the term "30 min" will be used as a general descriptor for samples collected at 20/30/40 min, since the data were scarce for independent evaluation in each time. Prior to estimation of the pooled estimate mean and SD for vocalisation, the data were submitted to logarithmic transformation according to techniques for separate standard deviations proposed by Higgins *et al.* (2008). The random effect MA and meta-regression were carried out given a priori assumption of between-study heterogeneity (DerSimonian and Laird, 1986)

The comparison group analysis was conducted on stratified subsets of data consisting of at least two individual studies that investigated similar treatments and had the same outcome. Many authors showed that this type of analysis with small number of trials are possible and the results are reliable (Mederos *et al.* 2012; Falzon *et al.* 2014; Lean *et al.* 2014). Simultaneously, we analysed each outcome separately as a group using stratification by dehorning technique and pain management. The results of MA were presented with the pooled MD and 95% CI. Cochran's Q (a chi-squared test of heterogeneity) and I^2 (percentage of total variation between studies that is due to heterogeneity rather than chance) were obtained based on the dehorning technique and outcome. Differences were considered significant at $P < 0.05$ and trends were defined at $0.05 \le P < 0.1$. The magnitude of I^2 was considered low, moderate or high heterogeneity when the values were in order of 25%, 50%, and 75%, respectively (Higgins *et al.* 2003).

Publication bias. We investigated the possibility of publication bias graphically (funnel plot) and statistically (Begg's adjusted rank correlation and Egger's regression asymmetry tests) for each outcome. Bias was considered based on visual plot and if at least one of the statistical methods was considered significant ($P < 0.10$). If there was any evidence, the "trim-and-fill" method was used to estimate and correct for an eventual publication bias (Duval and Tweedie 2000).

Meta-regression. Univariable random-effects analysis were performed to evaluate the effects of (1) randomization (no or yes), (2) cluster control (no, yes, or not applicable), (3) confounders identified and controlled (no, yes, or not applicable), (4) manuscript publication year, (5) publication type (peer-reviewed, conference proceedings, thesis, or government/research stations reports), (6) continent (North America, South America, Europe, Asia, or Oceania), (7) cattle group (*Bos taurus taurus*, *Bos taurus indicus*, hybrid/mixed, or not reported), (8) cattle sex (not reported, female, male, or mixed), (9) who performed the procedure (not reported, farm staff, or veterinarian), (10) application of pain relief (no or yes), (11) class of pain relief (not applicable, anaesthesia, NSAIDs, or multimodal therapy), (12) dehorning technique (amputation, cautery, or amputation *vs.* cautery), (13) cattle age (days), (14) intervention follow-up (days), and (15) sample size on each outcome of interest. The variables were analysed separately due to the low number of studies available for each outcome of interest.

Cumulative MA. Cumulative MA is frequently constructed of performing new MA every time the result of a potential new study is published. Then, the data are sorted chronologically to identify any temporal patterns in the results (Borenstein *et al.* 2009).

Influential studies. Studies influencing the heterogeneity and the MD were detected in the sensitivity analyses. This was performed by manually replacing and removing one study at a time and evaluating whether the mean difference had changed by more than 30%.

Results

Studies identified and information extracted

The literature search identified 1 248 citations. Of these, 102 were identified as useful manuscripts or reports likely to contain data, but only 33 were determined as eligible and were included for methodological soundness and data extraction (Fig. 1). For SR-MA, seven studies provided extractable data (Table 2).

(Insert Table 2 here)

From three contacted authors who presented their results graphically or without sufficient data, no numerical data were obtained. The data were then manually extracted.

The alternative treatments evaluated in the review were amputation (n = 6 studies) and cautery (n = 2 studies) dehorning. No quantitative analysis was done for amputation *vs.* cautery technique, since only one study reached the data extraction stage. Relevant pain relief included four studies that analysed anaesthesia, a further one evaluated NSAIDs, and two evaluated multimodal therapy. The total number of cattle for the studies that evaluated dehorning and cortisol concentration, ADG, and vocalisation were 283, 131, and 139, respectively.

In total, four publications were included in this SR-MA that comprised seven studies and 69 unique treatment comparisons. Table 3 lists the characteristics of included studies.

(Insert Table 3 here)

Risk of bias

The assessment of risk of bias using Cochrane criteria and the methodological assessment in the included studies are shown in Tables 4 and 5, respectively.

(Insert Table 4 here)

(Insert Table 5 here)

The performance bias was unclear in 100% of the studies that analysed vocalisation and ADG, and in 83.1% of studies that evaluated cortisol concentration. The approach to blinding of outcome assessor was not reported, making the risk of detection bias high for vocalisation. With respect to the risk of attrition bias, this domain was low for all the included studies.

Statistical analysis

Four publications[1] reporting control studies, describing seven studies and 69 trials were included in the MA. There were no exclusions due to lack of randomization procedures or lack of adjusting for clustering and confounders. The number of publications, studies, trials, and type of outcome measurements available for the statistical analyses are presented in Table 6.

(Insert Table 6 here)

[1] One publication can report more than one study, and each study is composed by one or more trials (comparisons).

Effect of dehorning on cortisol concentration. The cortisol concentration was the most commonly investigated outcome, and all included studies provided data for MA. However, the difference attributable to the heterogeneity was high (I^2 = 50.5%).

Amputation dehorning: Combining data from six studies (*n* = 31 trials) gave a MD of -0.219 nmol/L (95% CI -0.420, -0.049), suggesting significant changes (*P* = 0.032) favouring control group, and moderate heterogeneity between studies (I^2 = 41.2%; *P* = 0.010). Compared to not dehorned, the dehorned animals with no pain mitigation showed significant higher cortisol level at 30 min (*n* = 8 trials; *MD* = -0.767; 95% CI -1.099, -0.435; *P* = 0.000), as well as at 120 min (*n* = 2 trials; *MD* = -0.680; 95% CI -1.267, -0.093; *P* = 0.023) after procedure, with no heterogeneity between studies (Fig. 2). In three studies (*n* = 7 trials) no significant effect in cortisol concentration in dehorning with anaesthesia was found, regardless of control group, 30 min after procedure, and 0% heterogeneity between studies.

(Insert Fig. 2 here)

Cautery dehorning: Pooled results from two studies (*n* = 13 trials) showed no evidence of changes on the overall effect of cortisol level and high heterogeneity between studies (I^2 = 58.6%; *P* = 0.004). In our database, only one study was available for dehorning without pain relief, for anaesthesia, and for multimodal therapy, and so comparisons were not possible.

Effect of dehorning on ADG. The heterogeneity between studies was high (I^2 = 70.5%) for those that evaluated ADG data as an animal welfare indicator.

Amputation dehorning: In the three studies (*n* = 15 trials) that analysed amputation dehorning, there was consistent evidence of an overall effect on the ADG (*MD* = 0.487; 95% CI 0.080, 0.895; *P* = 0.019) and high heterogeneity between studies (I^2 = 70.5%). A stratified analysis from three studies (*n* = 4 trials) involving non-dehorning and dehorning with no pain relief produced a combined MD of 0.800 g/day (95% CI -0.306, 1.907) with high heterogeneity between studies (I^2 = 83.8%). The use of anaesthesia, reported in two studies (n = 5 trials), presented no effect on ADG, despite of high heterogeneity between these studies.

Effect of dehorning on vocalisation. The included studies that reported vocalisation showed high heterogeneity between studies (I^2 = 91.9%).

Amputation dehorning: The overall mean difference reported in three studies (n = 10 trials) was -0.210 (95% CI -0.972, 0.553), suggesting no evidence of changes and moderate heterogeneity between studies (I^2 = 37.2%; P = 0.111). The effect size was -0.929 (95% CI -1.973, 0.116; P = 0.081; n = 4 trials) when dehorned animals were compared to control groups, with low heterogeneity between studies (I^2 = 23.4%; P = 0.271). No significant differences and no heterogeneity between studies (n = 2 trials) were found between different methods of amputation dehorning without pain relief.

Publication bias. As shown above, our data were highly heterogeneous and the results should be carefully interpreted. Publication bias was not detected by inspection of funnel plot, as well as by statistical Egger's and Begg's tests, when evaluating cortisol level and vocalisation as outcomes. For ADG there was some evidence of publication bias. The visual inspection of the funnel suggested asymmetry, the adjusted rank correlation revealed a significant bias (P = 0.012), and the "trim-and-fill" method indicated that two additional studies have been necessary to balance the funnel plot.

Meta-regression. Seven studies (n = 69 trials) were included in the meta-regression analysis.

Meta-regression results for cortisol: Seven studies (n = 44 trials) were submitted to the univariable meta-regression analysis. Five of 15 considered variables explained 95% of the total variance (Table 7). Changes in cortisol concentration showed a direct association with the sample size. Only one variable related to study quality, recorded in the database, tended to show a significant association with the outcome of interest. Cortisol levels in studies published in theses tended to be lower than in those published in peer-reviewed journals. Studies evaluating dehorning with local anaesthesia or multimodal therapy had a significant effect on change in cortisol concentrations compared to dehorning with no pain relief.

(Insert Table 7 here)

Meta-regression results for ADG: None of the variables showed an association with ADG, nor contributed to explain the variation between studies, by the univariable meta-regression, which included three studies (n = 15 trials).

Meta-regression results for vocalisation: The univariable meta-regression was performed in three studies (n = 10 trials). None of the variables showed an effect on vocalisation. However, the use and the class of pain relief explained 100% of the total variance.

Cumulative MA. There was no evidence of change in the estimated point of the pooled treatments MD for cortisol levels; however, a pattern was observed over time. During the 1990s, a trial from Cooper *et al.* (1995) had the highest treatment effect (MD = -1.186 nmol/L), which tended to decline to -0.117 nmol/L in the 2013 (Hubber *et al.* 2013). Since all publications for ADG and vocalisation outcomes were published in 2012, we could not perform the analysis.

Influential studies. The pooled estimate for the impact of dehorning on cortisol levels showed a reduction from -0.117 nmol/L to -0.249 nmol/L by removing Hubber *et al.* (2013) of the analysis; and an increase to -0.061 nmol/L by omitting one study of Sinclair (2012). In addition, another study from Sinclair (2012) increased the MD to -0.071. The pooled estimate for the effects of dehorning on ADG showed an increase and a reduction from 0.487 g/day to 0.656 g/day and to 0.237 g/day, respectively, by removing two studies from database (Sinclair, 2012). Finally, removing two studies from Sinclair (2012) thesis at a time changed the pooled estimate for the number of vocalisations' during the procedure from -0.289 to -0.745 and 0.343.

Discussion

The public concern about pain caused by routine husbandry practices in farm animals has increased in recent years (Stafford and Mellor 2005), since painful procedures, such as dehorning, can have a negative public perception (Stock *et al.* 2013).

In spite of the fact that literature focusing on pain management in cattle during dehorning is plentiful (Schwartzkopf-Genswein *et al.* 2005; Doherty *et al.* 2007; Stilwell *et al.* 2009; Sinclair 2012; Hubber *et al.* 2013), only a small number of publications were available for our SR-MA. One probable explanation is that many studies were performed in dairy cattle. Second, as dehorning causes pain-induced distress and may be eliminated from the farm, this procedure in beef cattle is decreasing. Finally, as more research is needed to continue to determine better indicators of pain (Stock *et al.* 2013), the choice of those three outcomes (cortisol level, ADG and vocalisation) may not have been the most appropriate.

From the seven studies providing data useful for MA, the majority was conducted in Australia or New Zealand during the 2000s. Several countries, including those in the European Union and Oceania, have been reviewing their dehorning welfare codes (Stock *et al.* 2013). The delay in developing methods of recognition and assessment of animal pain has been due to the unwillingness of some researchers to accept that animals are capable of suffering (Molony and Kent 1997). In addition, the approval and sustainability of new drugs for commercial use on production animals (Smith and Modric 2013) can explain the increase in publications in this century.

The effect of dehorning on cortisol concentration

Changes in physiology, such as cortisol and heart rate, following cattle dehorning are frequently used as biomarkers in pain assessment (Schwartzkopf-Genswein *et al.* 2005; Stock *et al.* 2013). Cortisol levels represent only one feature of an animal's stress response, excluding for instance more rapid sympathetico-adreno medullary response (Mellor and Stafford 1997). However, interpreting an animal's subjective experience using physiological indicators will always be difficult, since there are variables that can limit the use of this information for assessment of pain, including diurnal changes, sample collection and the wide variety of causes that can activate the stress response (Mellor and Stafford 1997; Molony and Kent, 1997; Möstl and Palme 2002). Furthermore, even though Stafford and Mellor (2005) reported that the individual responses were similar with small variances in most studies about dehorning, the inter-animal variations in the stress response should be accounted for (Mellor and Stafford, 1997; Molony and Kent 1997; Mellor *et al.* 2000). With the debate about the validity of using cortisol responses (Mellor and Stafford 1997) and few effective physiological alternatives (Stafford and Mellor 2005), several authors have investigated non-invasive sampling procedure for corticoid such as determination in urine, saliva, milk, or faeces (Möstl and Palme 2002).

Heterogeneity was observed in those studies that evaluated the effect of dehorning on cortisol concentration. Although those performing SR-MA included searches of dissertations to ensure comprehensive identification of all relevant studies (Egger *et al.* 2001), two influential studies were published in theses (Sinclair 2012), a factor that contributed to the variation in cortisol and explaining almost 15% of the total variance. The only study that used blinding of outcome assessment and had the largest sample size (n = 79 animals) was published by Hubber *et al.* (2013). These variables together contributed with more than 30% of the total variance and in cortisol response. Careful design, conduct, and analysis of a trial prevents detection bias (Egger *et al.* 2001). As a consequence of the variation between animals, the stress response decreases our capacity to detect differences among groups and greater number of animals are required (Mellor *et al.* 2000). Mellor and Stafford (1997) suggested that with larger group numbers, the differences among treatments might have become significant.

In this MA, the response of cortisol secretion to amputation dehorning with no pain relief was as expected. The qualitative nature of the distress caused by dehorning can be characterized in two phases of cortisol response. The first, an initial peak due to horn amputation, occurring after about 30 min, is followed by an inflammatory phase consisting of a plateau and subsequent decline to pre-treatment levels by 5-6 h after dehorning (Cooper *et al.* 1995; McMeekan *et al.* 1998; Mellor *et al.* 2002). Several studies observed an increase in cortisol concentration in response to dehorning (Cooper *et al.* 1995; Mellor *et al.* 2002; Sinclair 2012), despite the fact that calf distress responses vary, both between and within each method (McMeekan *et al.* 1997). The comparison between four methods of mechanical dehorning conclude that the maximum cortisol secretion occurs during the first hour (Sylvester *et al.* 1998*a*), with no difference in relation to the depth of the wound (McMeekan *et al.* 1997)

No effect of anaesthesia in decreasing cortisol concentration was observed in our SR-MA, despite showing that prior administration of local anaesthesia diminished the cortisol level exhibited by dehorned cattle during the first 2 h (McMeekan *et al.* 1998; Mellor *et al.* 2002; Sinclair 2012) and 3 h (Sylvester *et al.* 1998*b*) to the levels of the handled only calves. Our result was similar to the findings of Doherty *et al.* (2007), who demonstrated a peak in cortisol concentration within 30 min of treatment in control and treated groups. Moreover, there was no difference among groups for the area under the cortisol response curve (Sinclair, 2012). However, the administration of a local anaesthetic in conjunction with NSAID (McMeekan *et al.* 1998; Stilwell *et al.* 2012) or the combination of local anaesthetic and cauterising the dehorning wound (Sylvester *et al.* 1998*b*) can virtually abolish the delayed cortisol response. It is hoped that pain relief can be more freely available to farmers worldwide (Stafford and Mellor 2011).

Furthermore, meta-regression analyses suggested a significant increase in cortisol levels in dehorned animals with local anaesthesia. One probable explanation is that the injection *per se* before dehorning may confound the interpretation, not primarily due to the punctures itself, but presumably due to the pressure caused by the injected volumes (Graf and Senn 1999). Second, even though Schwartzkopf-Genswein *et al.* (2005) and Graf and Senn (1999) indicated that the handling and restraint associated with dehorning itself did not evoke an additional rise in hormone concentration, the increase can occur in animals unaccustomed to handling (Stafford and Mellor 2011;

Sinclair 2012). Third, differences exist in the method of anaesthesia. Most studies block only the perineural space surrounding the cornual nerve (a branch of the Trigemial nerve, cranial nerve V) (Morisse *et al.* 1995; McMeekan *et al.* 1998; Mellor *et al.* 2002), whereas others attempted to completely desensitize other local nerve blocks, such as ring blocks or caudal horn blocks (Graf and Senn 1999; Faulkner and Weary 1997; Doherty *et al.* 2007; Sinclair 2012). Morisse *et al.* (1995) showed that the effectiveness of anaesthesia was obvious in only 60% of animals in the experiment. Finally, the *ceiling effect* on cortisol secretion can suppress further increases with the more invasive treatments (Mellor *et al.* 2000).

When looking at all studies which analysed cautery dehorning, there was no consistent evidence of an overall effect on the cortisol levels. A summary effect calculation by the pain relief classes would be invalid here as there was not sufficient data to obtain a clear conclusion. The transient increase in cortisol concentration was normally reduced by the administration of local anaesthetic (Mellor and Stafford 1997) or multimodal therapy (Hubber *et al.* 2013), suggesting that the pain relief can reduce the cortisol to baseline levels. However, when hot-iron dehorning was performed without pain relief, the increase in cortisol response was greater by 30 min (Sinclair 2012), 60 min (Stilwell *et al.* 2012), and 120 min (Schwartzkopf-Genswein *et al.* 2005) post-treatment than in the sham-dehorned group. Moreover, subtle differences in technique may account for reported differences across studies using thermal dehorning (Doherty *et al.* 2007). As concluded by Graf and Senn (1999), cattle experienced considerable stress and pain by heat cauterization, with a moderate (55%) overall acute cortisol response (Stafford and Mellor 2005).

The pattern observed in the cumulative meta-analysis might be related to a combination of several factors, such as an improvement in study design; in the 2000s, the literature focusing on the use of analgesic regimens following dehorning such as NSAIDs, anaesthesia, and sedatives with analgesic properties is plentiful (Stafford and Mellor 2005; Stock *et al.* 2013); and more precise assessment tools used to determine the efficacy with analgesic drugs in cattle following dehorning (Stock *et al.* 2013). However, the effect might have been confounded by other factors, which did not show any significant association (e.g., age, breed, gender) or it was not controlled for (e.g., horn size, tissue damage) with cortisol concentration in our SR-MA.

The effect of dehorning on ADG

Research to date on pain assessment in animals can also measure general body function, or production variables, such as bodyweight and food intake (Weary *et al.* 2006). Moreover, whether economic gains could balance the cost, pain management at the time of dehorning might be adopted more readily by producers (Newton and O'Connor 2013; Stock *et al.* 2013). However, the use of ADG as a painful biomarker is not common, as we could see in this SR.

In agreement with our results, Sinclair (2012) and Neely *et al.* (2014) observed no effect on ADG after amputation dehorning in comparison to non-dehorned cattle. Even though amputation dehorning decreased grazing behaviour and increased restlessness, there was no difference in the appetite score nor in food intake (Sylvester *et al.* 2004; Sinclair 2012; Neely *et al.* 2014). Sinclair (2012) demonstrated that there is a response to the stress on treatment day, whereby feeding is suppressed to begin with and replaced by locomotion, confirmed by the reduction in ADG at two weeks post-dehorning. It is reasonable to assume that the difference in the behaviour, together with cortisol changes, suggests that dehorning causes significant pain in the first 6 h (Sylvester *et al.* 2004).

We observed a similar pattern when dehorned cattle received anaesthesia. As suggested by Sylvester *et al.* (2004), during the period of anaesthesia (2 h), differences in the daily feed intake and some behavioural differences, including rumination (Newton and O'Connor, 2013), can be eliminated. On the other hand, the use of NSAIDs can affect the performance and feeding behaviour of calves after cautery (Faulkner and Weary 2000) and amputation dehorning (Sinclair 2012). Some of the differences in feeding behaviour, not in ADG *per se*, may not be an effect of the pain relief itself, but may be a consequence of the drug's effect.

A critical examination for the presence of publication bias, and other reporting biases, is crucial in the MA process (Egger *et al.* 2001). The *funnel plot,* as well as the results from Begg's test and "trim-and-fill" method, indicated a publication bias. Additional studies under commercial conditions would be recommended to address the long-term potential performance impacts of dehorning. Therefore, reporting guidelines for randomized controlled trials, which Sargeant et al. (2005) published, can help the authors to provide complete and accurate details of the methods used in the trials.

The average effect changed after the removal two studies published by Sinclair (2012). The effect increased by 35% in one study and decreased by 51% in the other, but still remained positive. These studies had a relatively small sample size per group (n = 9 to 13 cattle), and the precision of estimates was high, which may influence the average effect. Furthermore, a relevant point is the observation period for this outcome (13 and 56 days), since long-term impact of dehorning in ADG is the important question (Newton and O'Connor 2013).

The effect of dehorning on vocalisation

Veterinary and animal science professionals have used behavioural assessments of pain since their inception (Schwartzkopf-Genswein *et al.* 2012). Pain-related behaviours can be good indicators of the duration and the different phases of a painful experience (Stafford and Mellor 2005). It was highlighted by Stilwell *et al.* (2009) that behaviour analysis is a better indicator of a very recent pain-induced distress possibly because the cortisol response is delayed. In addition, it can be seen immediately, allowing speedy assessment (Mellor *et al.* 2000). Important behavioural indicators of pain for dehorning management include vocalisations, head shakes, head rubs, ear flicks, and tail flicks (Molony and Kent 1997; Stock *et al.* 2008).

The dehorned cattle showed a tendency to vocalise more often than non-dehorned cattle. This increase in the number of vocalisations have previously been associated with greater pain during dehorning (Schwartzkopf-Genswein *et al.* 2005). Neely *et al.* (2014) observed that mechanical dehorning had greater vocalisation scores and more extended vocalisation than sham dehorned. Although injected local anaesthetic reduced vocalisations at dehorning, a topical anaesthetic was not effective (Sinclair 2012). Moreover, those animals that received local anaesthetic and NSAID vocalised fewer times during dehorning than without pain relief (Sinclair 2012). Traditionally, amputation wounds were cauterised to reduce haemorrhage (Stafford and Mellor 2011); however, during dehorning, the animals that received topical anaesthetic and had their horn buds cauterized showed significantly more counts of vocalisation, and greater inflammation, tissue damage and slower wound healing rates (Sinclair 2012). A marked increase in other behaviours, such as forcing ahead, rearing and struggling, is strong evidence of avoidance and escape, which is apparently indicative of pain and stress after dehorning, regardless of the instrument used (Graf and Senn 1999; Sinclair 2012).

Although Neely *et al.* (2014) observed significant differences in the vocalisation score between two different amputation dehorning techniques in cattle, we did not find differences on the number of vocalisations. Sinclair (2012) showed no differences between knife and scoop dehorner and these groups vocalised more than animals dehorned with a hot-iron. Additionally, there were no differences for this behaviour if local anaesthetic (Doherty *et al.* 2007) or NSAID (Faulkner and Weary 2000) were used prior to hot-iron dehorning.

Even though two of the three studies included in our SR-MA showed an immediate influence, speculations about reasons for differences in vocalisation did not show any significant effect. Nevertheless, these analyses would have had limited power given the small number of trials available (Borenstein *et al.* 2009). Furthermore, in the manner vocalisation was measured, the potential for detection bias was high. This suggests that larger, well-reported field studies are needed to validate this behaviour as an indicator of pain.

Our SR-MA has limitations. First, the approach to reporting outcomes often limited our ability to summarize the data, since there was incomplete reporting of summary measures; therefore, an attempt was made by contacting researchers in the field (Egger *et al.* 2001). Second, we had to exclude 10 full-text publications on dehorning or disbudding because they were written in German, Norwegian, or Japanese, which might have introduced language bias, since negative findings are published in local journals, i.e. non-English-language reports (Egger *et al.* 2001). Finally, with the lack of pain-specific measures, the choice of indicators of welfare and its relationship on the dehorning may be difficult.

In conclusion, this is the first SR-MA that summarized the available literature on the effects of dehorning on beef cattle welfare. We demonstrated that dehorning reduces the welfare of beef cattle by the increase in cortisol concentration and in the number of vocalisations; however, did not change the ADG. Local anaesthesia did not reduce pain-induced distress, measured by cortisol level, following dehorning. The challenges on this subject are: conduct research on effective strategies to alleviate the stress and pain experienced by dehorned cattle; validate an improved physiological biomarker of pain; and considerate that the genetic control is possible to decline this undesirable characteristic, but the results can only be seen in the long term (Stafford and Mellor 2011; Stock *et al.* 2013).

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Table 1. Population, outcome and intervention search term strings used for the final search in the systematic review

Acronym	Search string
Population	Bovine: refers to the subfamily Bovinae, which includes cattle, buffalo, and kudus. Beef cattle: are the domestic cattle to produce meat. Calf: as a young female or male bovine up to weaning. Herd: a group of animals that live or are kept together.
Intervention	Disbudding: refers to prevention of horn growth before it has become advanced. Dehorning: the amputation of horns at any stage after their growth of the early budding stage. Castration: is the process of removal, damage, or destruction of the testicles.
Outcome	Animal welfare or animal well-being: involves basic health and functioning, natural living and affective state. Animal pain: is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is describable in terms of such damage. Animal stress: biological response elicited when an individual perceives a stressor to its homeostasis. Cortisol: widely used as a hormonal indicator of pain-induced distress caused by a range of husbandry practices in farm animals. In response to emotionally and physically noxious experiences, there is an increase in the activity of the hypothalamic-pituitary-adrenocortical system, i.e. in the cortisol level. Behaviour: farm animal welfare behaviour has been used to assess the response to painful husbandry procedures. Behavioural indicators, measured objectively or subjectively, can provide robust assessment tools for pain with that they are clearly explained and validated. Vocalisation: vocalisation may well be a good behavioural indicator of pain (Watts and Stookey 2000). Hence, researchers are interested in using vocal behavior in farm animals as a way to evaluate their welfare.

Table 2. A descriptive summary of each relevant study included in the meta-analysis and meta-regression (7)

Reference	Publication type	Country	Study population (age in days / sample size)	Procedure	Analgesic regimen	Outcome parameter
Cooper *et al.* 1995	Peer-reviewed	Canada	180 / 12	Amputation dehorning	NA	Cortisol (30 minutes)
Mellor *et al.* 2002	Peer-reviewed	New Zealand	70 / 30	Amputation dehorning	Local anaesthesia	Cortisol (30 and 120 minutes)
Sinclair 2012	Thesis	Australia	217 / 56	Amputation dehorning	NSAID and multi-modal therapy	Cortisol (30 and 120 minutes) Vocalisation (during dehorning)
Sinclair 2012	Thesis	Australia	217 / 27	Amputation dehorning	Local anaesthesia	Cortisol (30 minutes) ADG (56 days)
Sinclair 2012	Thesis	Australia	232 / 48	Amputation dehorning	Local anaesthesia	Cortisol (30 minutes) ADG (13 days) Vocalisation (during dehorning)
Sinclair 2012	Thesis	Australia	120 / 35	Amputation and cautery dehorning	NA	Cortisol (30 minutes) Vocalisation (during dehorning)
Hubber *et al.* 2013	Peer-reviewed	Austria	210 / 79	Cautery dehorning	Local anaesthesia and multi-modal therapy	Cortisol (30 and 120 minutes)

ADG: average daily gain; NSAID: non-steroidal anti-inflammatory drug; NA: not applicable.

Table 3. Descriptive characteristics of four publications reporting seven studies included in the systematic review-meta-analysis

Variable	Categories	Number of publications (studies)
Study design	Control studies	4 (7)
Publication type	Peer-reviewed	3 (3)
	Conference proceedings	0 (0)
	Thesis	1 (4)
	Government or research station report	0 (0)
Treatment (type of technique)	Amputation dehorning	3 (6)
	Cautery dehorning	2 (2)
	Amputation *vs.* Cautery dehorning	1 (1)
Data published	1990-2000	1 (1)
	2001-2015	3 (6)
Pain relief	No	3 (6)
	Yes	3 (5)
Class of pain relief	Local anaesthesia	3 (4)
	NSAID	1 (1)
	Multi-modal therapy	2 (2)
Cattle sex	Female	1 (3)
	Male	1 (1)
	Female and male	2 (2)
	Not reported	1 (1)
Cattle group	*Bos taurus taurus*	1 (1)
	Bos taurus indicus	0 (0)
	Hybrid / Mixed	2 (5)
	Not reported	1 (1)
Who performed the procedure	Farm staff	1 (3)
	Veterinarian	0 (0)
	Not reported	4 (4)
Outcome assessed	Average daily gain	1 (3)
	Cortisol concentration	4 (7)
	Vocalisation	1 (3)
Sample size	n≤50	3 (5)
	n= 51-100	2 (2)
Continent	North America	1 (1)
	South America	0 (0)
	Europe	1 (1)
	Asia	0 (0)
	Oceania	2 (5)

NSAID: non-steroidal anti-inflammatory drug

Table 4. Internal validity of the seven included studies in the systematic review of welfare in dehorned beef cattle using the Cochrane Collaboration tool for assessing risk of bias

Reference	Sequence generation	Allocation concealment	Selective reporting	Outcome measurement	Blinding of personnel	Blinding of outcome assessment	Incomplete outcome data
Cooper *et al.* 1995	High	High	Low	Cortisol	Unclear	Low	Low
Mellor *et al.* 2002	Low	Unclear	Low	Cortisol	Unclear	Low	Low
Sinclair 2012	Low	High	Low	Cortisol ADG Vocalisation	Unclear Unclear Unclear	Low Low High	Low Low Low
Sinclair 2012	Low	High	Low	Cortisol ADG	Unclear Unclear	Low Low	Low Low
Sinclair 2012	Low	High	Low	Cortisol ADG Vocalisation	Unclear Unclear Unclear	Low Low High	Low Low Low
Sinclair 2012	High	High	High	Cortisol Vocalisation	Unclear Unclear	Low High	Low Low
Hubber *et al.* 2013	Low	Low	Low	Cortisol	Low	Low	Low

ADG: average daily gain

Table 5. Summary of assessment for methodological soundness and/or reporting of four publications reporting seven studies including in this review

		Number of publications (studies)		
Variable	Assessment	ADG	Cortisol	Vocalisation
Was the sample size justified?	Yes	0 (0)	0 (0)	0 (0)
	No	1 (3)	4 (7)	1 (3)
How were calves assigned to treatment groups?	Random[A]	0 (0)	1 (1)	0 (0)
	Reported random[B]	1 (3)	2 (4)	2 (2)
	Systematic[C]	0 (0)	0 (0)	0 (0)
	Convenience or unreported[D]	0 (0)	2 (2)	1 (1)
Was the intervention protocol described in sufficient detail to be replicated?	Yes	1 (3)	2 (5)	1 (3)
	No	0 (0)	2 (2)	0 (0)
	Reference paper	0 (0)	0 (0)	0 (0)
Did the author report that blinding was used to evaluate the outcome?	Yes	0 (0)	1 (1)	0 (0)
	No	1 (3)	3 (6)	1 (3)
Based on the study design was clustering[E] accounted for appropriately in the analysis?	Yes	1 (3)	3 (6)	1 (3)
	No	0 (0)	1 (1)	0 (0)
	Not applicable	0 (0)	0 (0)	0 (0)
Were identified confounders controlled for or tested?	Yes, analysis[F]	0 (0)	0 (0)	0 (0)
	Yes, inclusion/exclusion[G]	1 (3)	2 (5)	1 (3)
	Yes, matching[H]	0 (0)	0 (0)	0 (0)
	No[I]	0 (0)	1 (1)	0 (0)
	Not applicable[J]	0 (0)	1 (1)	0 (0)
Was the statistical analysis described adequately so it can be reproduced?	Yes	1 (3)	3 (6)	1 (3)
	No	0 (0)	1 (1)	0 (0)
	Reference paper	0 (0)	0 (0)	0 (0)
	Statistical analysis not done	0 (0)	0 (0)	0 (0)

ADG: average daily gain

[A]Computer or random number table, *a priori,* stratified random sample, cluster random sample.

[B]Author(s) report random, but randomization is not described.

[C]Taken n samples at interval of x or stratified by certain characteristics.

[D]Author indicated convenience sampling or sampling was not reported in the paper.

[E]Clustering was evaluated when repeated measures were reported.

[F]Author identified confounders and controlled for them in the analysis.

[G]Confounders were identified and included/excluded a priori.

[H]Confounders were controlled a priori by matching on certain characteristics.

[I]No adjustments were made for confounders/effect modifiers, etc., that were identified by the author.

[J]Confounders were not identified by the author or randomization was used to control for confounders.

Table 6. Number of publications and number of controls studies used in meta-analysis and/or meta-regression, considering technique, outcome, and the use of pain relief

		Studies (trials)		
	Publication (studies)	ADG	Cortisol	Vocalisation
Pain relief		*Amputation dehorning*		
No	3 (6)	3 (5)	6 (12)	2 (4)
Yes	2 (4)	3 (10)	4 (19)	2 (6)
Anaesthesia	2 (3)	2 (5)	3 (9)	1 (3)
NSAID	1 (1)	1 (1)	1 (4)	1 (1)
Multimodal therapy	1 (1)	1 (4)	1 (6)	1 (2)
Total	*3 (6)*	*3 (15)*	*6 (31)*	*3 (10)*
		Cautery dehorning		
No	1 (1)	0 (0)	1 (1)	0 (0)
Yes	1 (1)	0 (0)	1 (12)	0 (0)
Anaesthesia	1 (1)	0 (0)	1 (2)	0 (0)
NSAID	0 (0)	0 (0)	0 (0)	0 (0)
Multimodal therapy	1 (1)	0 (0)	1 (10)	0 (0)
Total	*2 (2)*	*0 (0)*	*2 (13)*	*0 (0)*

ADG: average daily gain; NSAID: non-steroidal anti-inflammatory drug.

Table 7. Results from univariate meta-regression showing significant (*P* < 0.05) and marginally significant (0.05 ≤ *P* < 0.1) covariates investigated as potentials sources of study heterogeneity. The results explained for each of the covariates included in the meta-analysis are presented for cortisol concentration as an outcome

No. studies[A] (trials)[B]	Covariate (trials)	Estimate[C]	95% CI[D]	p-value	I^2 (%)	Adj-R^2 (%)
Cortisol 7 (44)						
	Null model	-0.10	-0.29, 0.07	0.244	54.10	NA
	Sample size (n = 44)	0.02	-0.0004, 0.042	0.046	50.60	15.08
	Blinding outcome assessment				50.55	16.37
	Yes (n = 12)	Referent				
	No (n = 32)	-0.37	-0.75, 0.01	0.057		
	Publication type				51.31	13.73
	Peer-reviewed (n = 19)	Referent				
	Thesis (n = 25)	-0.30	-0.67, 0.05	0.096		
	Continent			0.0806[E]	50.36	18.56
	North America (n = 1)	Referent				
	Europe (n = 12)	1.33	-0.30, 2.96			
	Oceania (n = 31)	0.97	-0.64, 2.59			
	Class of pain relief			0.0185	46.28	31.50
	Not applicable (n = 13)	Referent				
	Anaesthesia (n = 11)	0.63	0.15, 1.11	0.011		
	NSAID (n = 4)	0.10	-0.55, 0.75			
	Multimodal therapy (n = 16)	0.59	0.17, 1.01	0.007		

I^2: between-study residual variation; Adj-R^2: percentage of the residual variation; NSAID: non-steroidal anti-inflammatory drug.

[A]Number of studies included in the meta-regression.

[B]Number of trials included in the meta-regression.

[C]Standard mean difference of the effect size.

[D]These values represent 95% confidence intervals (CI) for the effect size.

[E]Significance of the categorical variable as a whole.

Fig. 1. Flow diagram outlining the screening process for the review of dehorning effects on welfare indicators. MA: meta-analysis. Adapted from PRISMA guidelines (Moher *et al.* 2009).

*Data from both procedures (castration and dehorning) are presented in the flow diagram to allow the researchers update this systematic review.

Fig. 2. Forest plot of studies that analysed the effect of amputation dehorning with no pain relief (on the right) in comparison to non-dehorned or dehorning by amputation without pain relief (on the left) at 30 min (a) and to non-dehorned (on the left) at 120 min (b). The effect size (ES) is the mean difference between treated and control groups, expressed in cortisol concentration (nmol/L). Note: The size of the plotting symbol for the point estimate in each study is proportional to the weight that each trial contributes in the meta-analysis. The dashed line is the average effect of treatment obtained by the analysis, while the solid vertical line marks the value at which the treatment would have no effect. The overall estimate and the confidence interval are marked by a diamond (♦).