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Published in:
Animal Production Science

DOI:
[10.1071/AN17752](https://doi.org/10.1071/AN17752)

First published: 30/05/2018

Document Version
Peer reviewed version

[Link to publication](#)

Citation for published version (APA):
Canozzi, MEA., Mederos, A., Turner, SP., Manteca, X., McManus, C., Menegassi, SRO., & Barcellos, JOJ. (2018). Dehorning and welfare indicators in beef cattle - a meta analysis. *Animal Production Science*, 59(5), 801-814. <https://doi.org/10.1071/AN17752>

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

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16

17 **Welfare and dehorning in cattle: a meta-analysis**

18 **Abstract.** Dehorning is a common practice in cattle farming. Researchers suggest that
19 pain during dehorning can be mitigated, although there is no conclusive evidence about
20 the best technique and the best manner of pain relief. A systematic review-meta-
21 analysis was performed to clarify the effect of dehorning on welfare indicators (cortisol
22 concentration or average daily gain [ADG] or **vocalisation**) in beef cattle up to 12
23 months of age. Five electronic databases were systematically searched, as well as
24 conference proceedings and experts were contacted electronically. Pre-defined
25 protocols were applied during all steps of the systematic review process. A random
26 effect meta-analysis was conducted for each indicator separately with the mean of the
27 control and treated groups. Four publications reporting 7 studies and 69 trials were
28 included in the MA involving 287 cattle. Heterogeneity between studies was observed
29 for cortisol ($I^2 = 50.5\%$), ADG ($I^2 = 70.5\%$), and **vocalisation** ($I^2 = 91.9\%$). When
30 comparing the non-dehorned group with amputation dehorning, **the** cortisol
31 concentration was lower 30 min ($P < 0.0001$) and 120 min ($P = 0.023$) after procedure
32 **(0.767 nmol/L and 0.680 nmol/L, respectively)**. Local anaesthesia did not show a
33 reduction in cortisol concentration at 30 min after dehorning by amputation. Non-
34 dehorned animals had a tendency to decrease the number of **vocalisation** ($P = 0.081$;
35 $MD = 0.929$) compared with the group dehorned by amputation. These results suggest
36 that dehorning is a painful experience and that local anaesthesia did not alleviate short-
37 term pain following dehorning. Further investigation into pain relief is required to
38 improve confident decision making under practical conditions.

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

40

41 **Introduction**

42 The prevention of horn growth (disbudding) or removal of horns (dehorning) are
43 **commonly** performed practices in the beef cattle industry (Stafford and Mellor 2005).
44 Regardless of the technique, disbudding and dehorning generate a pain-induced
45 response, which can be alleviated by applying strategies to alter the threshold of pain

46 or decrease the transmission of impulse in pain nerves from the wound (Sylvester *et al.*
47 1998*b*). Despite the evidence, the procedures are often performed without
48 administering analgesics (Stewart *et al.* 2009; Theurer *et al.* 2012). The recognition and
49 assessment of pain following painful procedures through a combination of
50 physiological, behavioural and production responses have been recommended
51 (Stafford and Mellor 2005).

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

58 The literature focusing on pain management in cattle during dehorning and
59 disbudding is plentiful (McMeekan *et al.* 1998; Schwartzkopf-Genswein *et al.* 2005;
60 Doherty *et al.* 2007; Sinclair, 2012; Hubber *et al.* 2013). The current state of knowledge
61 about **these** procedures and their relationship with pain alleviation have been
62 discussed subjectively in traditional reviews (Stafford and Mellor 2005, 2011).

63 However, it is crucial clarify the technique which causes the least pain and the best
64 pain relief to minimize pain-induced distress (Stafford and Mellor 2011; Vickers *et al.*
65 2005; Theurer *et al.* 2012). Hence, due the variability and difficulties in field research,
66 the systematic review (SR) and meta-analysis (MA), by integrating the findings from
67 many studies, can synthesize and increase the credibility of the results, providing a
68 more robust estimate of effect (Egger *et al.* 2001; Borenstein *et al.* 2009).

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

74 procedures, and the efficacy of pain relief on beef cattle welfare using a SR-MA
75 approach.

76

77 **Material and methods**

78 *Data source and searches*

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

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

102 of comparison used. **Vocalisation**, cortisol, and average daily gain (ADG) were the
103 interest outcomes.

104 (Insert Fig. 1 here)

105 (Insert Table 1 here)

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

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

113

114 *Selection of papers*

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

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

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

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

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

132

133 *Data extraction strategy and manipulation*

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

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

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

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

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

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

167 When the results were reported in the log-transformed scales, these were
168 transformed back to the original scale using the formula described by Mederos *et al.*
169 (2012). A pooled standard deviation (S_p) was based on the formula when an overall
170 standard error of the mean (SEM_p) was mentioned for the control and treatment
171 groups (Ceballos *et al.* 2009; Higgins and Green 2011; Mederos *et al.* 2012):

$$172 \quad S_p = SEM_p \times \sqrt{n_p}$$

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

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

$$178 \quad S_p = \frac{(x_2 - x_1)}{t(\alpha d f E) \sqrt{(1/n_2) + (1/n_1)}}$$

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

181 Additional considerations in the data-extraction step were as follows: when results
182 were presented as graphics, the corresponding author was contacted by electronic mail

183 and asked to provide the summary statistics. If no response was obtained or data were
184 not provided, the mean and/or measure of dispersion were manually extracted using a
185 ruler. Since the cortisol data were collected in three different times, the summary data
186 were recreated and the effect size was computed according to recommended
187 approaches (Borenstein *et al.* 2009).

188

189 *Assessment of risk of bias*

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

198

199 *Statistical analysis*

200 The Stata statistical package (version 14, StataCorp., College Station, TX, USA) was
201 used to analyse each outcome by mean difference (MD) between control and treatment
202 groups with a 95% confidence interval (95% CI). Data analysed for cortisol were
203 obtained from baseline to 20/30/40 min and up to 120 min; for ADG, during the follow-
204 up period reported by the authors; and for **vocalisation**, during the dehorning or
205 disbudding. For cortisol, the term “30 min” will be used as a general descriptor for
206 samples collected at 20/30/40 min, since the data were scarce for independent
207 evaluation in each time. Prior to estimation of the pooled estimate mean and SD for
208 **vocalisation**, the data were submitted to logarithmic transformation according to
209 techniques for separate standard deviations proposed by Higgins *et al.* (2008). The

210 random effect MA and meta-regression were carried out given a priori assumption of
211 between-study heterogeneity (DerSimonian and Laird, 1986)

212 The comparison group analysis was conducted on stratified subsets of data
213 consisting of at least two individual studies that investigated similar treatments and had
214 the same outcome. Many authors showed that this type of analysis with small number
215 of trials are possible and the results are reliable (Mederos *et al.* 2012; Falzon *et al.*
216 2014; Lean *et al.* 2014). Simultaneously, we analysed each outcome separately as a
217 group using stratification by dehorning technique and pain management. The results of
218 MA were presented with the pooled MD and 95% CI. Cochran's Q (a chi-squared test
219 of heterogeneity) and I^2 (percentage of total variation between studies that is due to
220 heterogeneity rather than chance) were obtained based on the dehorning technique
221 and outcome. Differences were considered significant at $P < 0.05$ and trends were
222 defined at $0.05 \leq P < 0.1$. The magnitude of I^2 was considered low, moderate or high
223 heterogeneity when the values were in order of 25%, 50%, and 75%, respectively
224 (Higgins *et al.* 2003).

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

231 *Meta-regression.* Univariable random-effects analysis were performed to evaluate the
232 effects of (1) randomization (no or yes), (2) cluster control (no, yes, or not applicable),
233 (3) confounders identified and controlled (no, yes, or not applicable), (4) manuscript
234 publication year, (5) publication type (peer-reviewed, conference proceedings, thesis,
235 or government/research stations reports), (6) continent (North America, South America,
236 Europe, Asia, or Oceania), (7) cattle group (*Bos taurus taurus*, *Bos taurus indicus*,
237 hybrid/mixed, or not reported), (8) cattle sex (not reported, female, male, or mixed), (9)

238 who performed the procedure (not reported, farm staff, or veterinarian), (10) application
239 of pain relief (no or yes), (11) class of pain relief (not applicable, anaesthesia, NSAIDs,
240 or multimodal therapy), (12) dehorning technique (amputation, cautery, or amputation
241 vs. cautery), (13) cattle age (days), (14) intervention follow-up (days), and (15) sample
242 size on each outcome of interest. The variables were analysed separately due to the
243 low number of studies available for each outcome of interest.

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

247 *Influential studies.* Studies influencing the heterogeneity and the MD were detected in
248 the sensitivity analyses. This was performed by manually replacing and removing one
249 study at a time and evaluating whether the mean difference had changed by more than
250 30%.

251

252 **Results**

253 *Studies identified and information extracted*

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

258 (Insert Table 2 here)

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

262 The alternative treatments evaluated in the review were amputation ($n = 6$ studies)
263 and cautery ($n = 2$ studies) dehorning. No quantitative analysis was done for
264 amputation vs. cautery technique, since only one study reached the data extraction
265 stage. Relevant pain relief included four studies that analysed anaesthesia, a further

266 one evaluated NSAIDs, and two evaluated multimodal therapy. The total number of
267 cattle for the studies that evaluated dehorning and cortisol concentration, ADG, and
268 vocalisation were 283, 131, and 139, respectively.

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

272 (Insert Table 3 here)

273

274 *Risk of bias*

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

277 (Insert Table 4 here)

278 (Insert Table 5 here)

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

284

285 *Statistical analysis*

286 Four publications¹ reporting control studies, describing seven studies and 69 trials were
287 included in the MA. There were no exclusions due to lack of randomization procedures
288 or lack of adjusting for clustering and confounders. The number of publications,
289 studies, trials, and type of outcome measurements available for the statistical analyses
290 are presented in Table 6.

291 (Insert Table 6 here)

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

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

295 Amputation dehorning: Combining data from six studies ($n = 31$ trials) gave a MD of
296 -0.219 nmol/L (95% CI $-0.420, -0.049$), suggesting significant changes ($P = 0.032$)
297 favouring control group, and moderate heterogeneity between studies ($I^2 = 41.2\%$; $P =$
298 0.010). Compared to not dehorned, the dehorned animals with no pain mitigation
299 showed significant higher cortisol level at 30 min ($n = 8$ trials; $MD = -0.767$; 95% CI -
300 $1.099, -0.435$; $P = 0.000$), as well as at 120 min ($n = 2$ trials; $MD = -0.680$; 95% CI -
301 $1.267, -0.093$; $P = 0.023$) after procedure, with no heterogeneity between studies (Fig.
302 2). In three studies ($n = 7$ trials) no significant effect in cortisol concentration in
303 dehorning with anaesthesia was found, regardless of control group, 30 min after
304 procedure, and 0% heterogeneity between studies.

305 (Insert Fig. 2 here)

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

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

313 Amputation dehorning: In the three studies ($n = 15$ trials) that analysed amputation
314 dehorning, there was consistent evidence of an overall effect on the ADG ($MD = 0.487$;
315 95% CI $0.080, 0.895$; $P = 0.019$) and high heterogeneity between studies ($I^2 = 70.5\%$).
316 A stratified analysis from three studies ($n = 4$ trials) involving non-dehorning and
317 dehorning with no pain relief produced a combined MD of 0.800 g/day (95% CI $-0.306,$
318 1.907) with high heterogeneity between studies ($I^2 = 83.8\%$). The use of anaesthesia,

319 reported in two studies ($n = 5$ trials), presented no effect on ADG, despite of high
320 heterogeneity between these studies.

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

323 Amputation dehorning: The overall mean difference reported in three studies ($n = 10$
324 trials) was -0.210 (95% CI $-0.972, 0.553$), suggesting no evidence of changes and
325 moderate heterogeneity between studies ($I^2 = 37.2\%$; $P = 0.111$). The effect size was $-$
326 0.929 (95% CI $-1.973, 0.116$; $P = 0.081$; $n = 4$ trials) when dehorned animals were
327 compared to control groups, with low heterogeneity between studies ($I^2 = 23.4\%$; $P =$
328 0.271). No significant differences and no heterogeneity between studies ($n = 2$ trials)
329 were found between different methods of amputation dehorning without pain relief.

330 *Publication bias.* As shown above, our data were highly heterogeneous and the results
331 should be carefully interpreted. Publication bias was not detected by inspection of
332 funnel plot, as well as by statistical Egger's and Begg's tests, when evaluating cortisol
333 level and **vocalisation** as outcomes. For ADG there was some evidence of publication
334 bias. The visual inspection of the funnel suggested asymmetry, the adjusted rank
335 correlation revealed a significant bias ($P = 0.012$), and the "trim-and-fill" method
336 indicated that two additional studies have been necessary to balance the funnel plot.
337 *Meta-regression.* Seven studies ($n = 69$ trials) were included in the meta-regression
338 analysis.

339 Meta-regression results for cortisol: Seven studies ($n = 44$ trials) were submitted to
340 the univariable meta-regression analysis. Five of 15 considered variables explained
341 95% of the total variance (Table 7). Changes in cortisol concentration showed a direct
342 association with the sample size. Only one variable related to study quality, recorded in
343 the database, tended to show a significant association with the outcome of interest.
344 Cortisol levels in studies published in theses tended to be lower than in those published
345 in peer-reviewed journals. Studies evaluating dehorning with local anaesthesia or

346 multimodal therapy had a significant effect on change in cortisol concentrations
347 compared to dehorning with no pain relief.

348 (Insert Table 7 here)

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

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

356 *Cumulative MA.* There was no evidence of change in the estimated point of the pooled
357 treatments MD for cortisol levels; however, a pattern was observed over time. During
358 the 1990s, a trial from Cooper *et al.* (1995) had the highest treatment effect ($MD = -$
359 1.186 nmol/L), which tended to decline to -0.117 nmol/L in the 2013 (Hubber *et al.*
360 2013). Since all publications for ADG and vocalisation outcomes were published in
361 2012, we could not perform the analysis.

362 *Influential studies.* The pooled estimate for the impact of dehorning on cortisol levels
363 showed a reduction from -0.117 nmol/L to -0.249 nmol/L by removing Hubber *et al.*
364 (2013) of the analysis; and an increase to -0.061 nmol/L by omitting one study of
365 Sinclair (2012). In addition, another study from Sinclair (2012) increased the MD to -
366 0.071 . The pooled estimate for the effects of dehorning on ADG showed an increase
367 and a reduction from 0.487 g/day to 0.656 g/day and to 0.237 g/day, respectively, by
368 removing two studies from database (Sinclair, 2012). Finally, removing two studies
369 from Sinclair (2012) thesis at a time changed the pooled estimate for the number of
370 vocalisations' during the procedure from -0.289 to -0.745 and 0.343 .

371

372 **Discussion**

373 The public concern about pain caused by routine husbandry practices in farm animals
374 has increased in recent years (Stafford and Mellor 2005), since painful procedures,
375 such as dehorning, can have a negative public perception (Stock *et al.* 2013).
376 In spite of the fact that literature focusing on pain management in cattle during
377 dehorning is plentiful (Schwartzkopf-Genswein *et al.* 2005; Doherty *et al.* 2007; Stilwell
378 *et al.* 2009; Sinclair 2012; Hubber *et al.* 2013), only a small number of publications
379 were available for our SR-MA. One probable explanation is that many studies were
380 performed in dairy cattle. Second, as dehorning causes pain-induced distress and may
381 be eliminated from the farm, this procedure in beef cattle is decreasing. Finally, as
382 more research is needed to continue to determine better indicators of pain (Stock *et al.*
383 2013), the choice of those three outcomes (cortisol level, ADG and **vocalisation**) may
384 not have been the most appropriate.

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

393

394 *The effect of dehorning on cortisol concentration*

395 Changes in physiology, such as cortisol and heart rate, following cattle dehorning are
396 frequently used as biomarkers in pain assessment (Schwartzkopf-Genswein *et al.*
397 2005; Stock *et al.* 2013). Cortisol levels represent only one feature of an animal's
398 stress response, excluding for instance more rapid sympathetico-adreno medullary
399 response (Mellor and Stafford 1997). However, interpreting an animal's subjective
400 experience using physiological indicators will always be difficult, since there are

401 variables that can limit the use of this information for assessment of pain, including
402 diurnal changes, sample collection and the wide variety of causes that can activate the
403 stress response (Mellor and Stafford 1997; Molony and Kent, 1997; Möstl and Palme
404 2002). Furthermore, even though Stafford and Mellor (2005) reported that the individual
405 responses were similar with small variances in most studies about dehorning, the inter-
406 animal variations in the stress response should be accounted for (Mellor and Stafford,
407 1997; Molony and Kent 1997; Mellor *et al.* 2000). With the debate about the validity of
408 using cortisol responses (Mellor and Stafford 1997) and few effective physiological
409 alternatives (Stafford and Mellor 2005), several authors have investigated non-invasive
410 sampling procedure for corticoid such as determination in urine, saliva, milk, or faeces
411 (Möstl and Palme 2002).

412 Heterogeneity was observed in those studies that evaluated the effect of dehorning
413 on cortisol concentration. Although those performing SR-MA included searches of
414 dissertations to ensure comprehensive identification of all relevant studies (Egger *et al.*
415 2001), two influential studies were published in theses (Sinclair 2012), a factor that
416 contributed to the variation in cortisol and explaining almost 15% of the total variance.
417 The only study that used blinding of outcome assessment and had the largest sample
418 size ($n = 79$ animals) was published by Hubber *et al.* (2013). These variables together
419 contributed with more than 30% of the total variance and in cortisol response. Careful
420 design, conduct, and analysis of a trial prevents detection bias (Egger *et al.* 2001). As a
421 consequence of the variation between animals, the stress response decreases our
422 capacity to detect differences among groups and greater number of animals are
423 required (Mellor *et al.* 2000). Mellor and Stafford (1997) suggested that with larger
424 group numbers, the differences among treatments might have become significant.

425 In this MA, the response of cortisol secretion to amputation dehorning with no pain
426 relief was as expected. The qualitative nature of the distress caused by dehorning can
427 be characterized in two phases of cortisol response. The first, an initial peak due to
428 horn amputation, occurring after about 30 min, is followed by an inflammatory phase

429 consisting of a plateau and subsequent decline to pre-treatment levels by 5-6 h after
430 dehorning (Cooper *et al.* 1995; McMeekan *et al.* 1998; Mellor *et al.* 2002). Several
431 studies observed an increase in cortisol concentration in response to dehorning
432 (Cooper *et al.* 1995; Mellor *et al.* 2002; Sinclair 2012), despite the fact that calf distress
433 responses vary, both between and within each method (McMeekan *et al.* 1997). The
434 comparison between four methods of mechanical dehorning conclude that the
435 maximum cortisol secretion occurs during the first hour (Sylvester *et al.* 1998a), with no
436 difference in relation to the depth of the wound (McMeekan *et al.* 1997)

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

449 Furthermore, meta-regression analyses suggested a significant increase in cortisol
450 levels in dehorned animals with local anaesthesia. One probable explanation is that the
451 injection *per se* before dehorning may confound the interpretation, not primarily due to
452 the punctures itself, but presumably due to the pressure caused by the injected
453 volumes (Graf and Senn 1999). Second, even though Schwartzkopf-Genswein *et al.*
454 (2005) and Graf and Senn (1999) indicated that the handling and restraint associated
455 with dehorning itself did not evoke an additional rise in hormone concentration, the
456 increase can occur in animals unaccustomed to handling (Stafford and Mellor 2011;

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

466 When looking at all studies which analysed cautery dehorning, there was no
467 consistent evidence of an overall effect on the cortisol levels. A summary effect
468 calculation by the pain relief classes would be invalid here as there was not sufficient
469 data to obtain a clear conclusion. The transient increase in cortisol concentration was
470 normally reduced by the administration of local anaesthetic (Mellor and Stafford 1997)
471 or multimodal therapy (Hubber *et al.* 2013), suggesting that the pain relief can reduce
472 the cortisol to baseline levels. However, when hot-iron dehorning was performed
473 without pain relief, the increase in cortisol response was greater by 30 min (Sinclair
474 2012), 60 min (Stilwell *et al.* 2012), and 120 min (Schwartzkopf-Genswein *et al.* 2005)
475 post-treatment than in the sham-dehorned group. Moreover, subtle differences in
476 technique may account for reported differences across studies using thermal dehorning
477 (Doherty *et al.* 2007). As concluded by Graf and Senn (1999), cattle experienced
478 considerable stress and pain by heat cauterization, with a moderate (55%) overall
479 acute cortisol response (Stafford and Mellor 2005).

480 The pattern observed in the cumulative meta-analysis might be related to a
481 combination of several factors, such as an improvement in study design; in the 2000s,
482 the literature focusing on the use of analgesic regimens following dehorning such as
483 NSAIDs, anaesthesia, and sedatives with analgesic properties is plentiful (Stafford and
484 Mellor 2005; Stock *et al.* 2013); and more precise assessment tools used to determine

485 the efficacy with analgesic drugs in cattle following dehorning (Stock *et al.* 2013).
486 However, the effect might have been confounded by other factors, which did not show
487 any significant association (e.g., age, breed, gender) or it was not controlled for (e.g.,
488 horn size, tissue damage) with cortisol concentration in our SR-MA.

489

490 *The effect of dehorning on ADG*

491 Research to date on pain assessment in animals can also measure general body
492 function, or production variables, such as bodyweight and food intake (Weary *et al.*
493 2006). Moreover, whether economic gains could balance the cost, pain management at
494 the time of dehorning might be adopted more readily by producers (Newton and
495 O'Connor 2013; Stock *et al.* 2013). However, the use of ADG as a painful biomarker is
496 not common, as we could see in this SR.

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

507 We observed a similar pattern when dehorned cattle received anaesthesia. As
508 suggested by Sylvester *et al.* (2004), during the period of anaesthesia (2 h), differences
509 in the daily feed intake and some behavioural differences, including rumination
510 (Newton and O'Connor, 2013), can be eliminated. On the other hand, the use of
511 NSAIDs can affect the performance and feeding behaviour of calves after cautery
512 (Faulkner and Weary 2000) and amputation dehorning (Sinclair 2012). Some of the

513 differences in feeding behaviour, not in ADG *per se*, may not be an effect of the pain
514 relief itself, but may be a consequence of the drug's effect.

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

522 The average effect changed after the removal two studies published by Sinclair
523 (2012). The effect increased by 35% in one study and decreased by 51% in the other,
524 but still remained positive. These studies had a relatively small sample size per group
525 ($n = 9$ to 13 cattle), and the precision of estimates was high, which may influence the
526 average effect. Furthermore, a relevant point is the observation period for this outcome
527 (13 and 56 days), since long-term impact of dehorning in ADG is the important question
528 (Newton and O'Connor 2013).

529

530 *The effect of dehorning on vocalisation*

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

540 The dehorned cattle showed a tendency to **vocalise** more **often** than non-dehorned
541 **cattle**. This increase in the number of **vocalisations** have previously been associated
542 with greater pain during dehorning (Schwartzkopf-Genswein *et al.* 2005). Neely *et al.*
543 (2014) observed that mechanical dehorning had greater vocalisation scores and more
544 extended **vocalisation** than sham dehorned. Although **injected local anaesthetic**
545 **reduced vocalisations** at dehorning, a topical anaesthetic was not effective (Sinclair
546 2012). Moreover, those animals that received local anaesthetic and NSAID **vocalised**
547 fewer times during dehorning than without pain relief (Sinclair 2012). Traditionally,
548 amputation wounds were cauterised to reduce haemorrhage (Stafford and Mellor
549 2011); however, during dehorning, the animals that received topical anaesthetic and
550 had their horn buds cauterized showed significantly more counts of **vocalisation**, and
551 greater inflammation, tissue damage and slower wound healing rates (Sinclair 2012). A
552 marked increase in other behaviours, such as forcing ahead, rearing and struggling, is
553 strong evidence of avoidance and escape, which is apparently indicative of pain and
554 stress after dehorning, regardless of the instrument used (Graf and Senn 1999; Sinclair
555 2012).

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

563 Even though two of the three studies included in our SR-MA showed an immediate
564 influence, speculations about reasons for differences in **vocalisation** did not show any
565 significant effect. Nevertheless, these analyses would have had limited power given the
566 small number of trials available (Borenstein *et al.* 2009). Furthermore, in the manner
567 **vocalisation** was measured, the potential for detection bias was high. This suggests

568 that larger, well-reported field studies are needed to validate this behaviour as an
569 indicator of pain.

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

579 In conclusion, this is the first SR-MA that summarized the available literature on the
580 effects of dehorning on beef cattle welfare. We demonstrated that dehorning reduces
581 the welfare of beef cattle by the increase in cortisol concentration and in the number of
582 vocalisations; however, did not change the ADG. Local anaesthesia did not reduce
583 pain-induced distress, measured by cortisol level, following dehorning. The challenges
584 on this subject are: conduct research on effective strategies to alleviate the stress and
585 pain experienced by dehorned cattle; validate an improved physiological biomarker of
586 pain; and considerate that the genetic control is possible to decline this undesirable
587 characteristic, but the results can only be seen in the long term (Stafford and Mellor
588 2011; Stock *et al.* 2013).

589

590 **Acknowledgments**

591 The help of Bárbara Bremm and Claudia Medeiros Camargo during screening the step
592 and all investigators who were willing to supply additional data during the systematic
593 review-meta-analysis process are gratefully appreciated. This study was supported by
594 The Brazilian Council of Scientific and Technological Development (CNPq / Project

595 166250/2015-5) and Coordination for the Improvement of Higher Education Personnel
596 (CAPES / Project PDSE 99999.012325/2013-09).

597

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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	<p>Bovine: refers to the subfamily Bovinae, which includes cattle, buffalo, and kudu.</p> <p>Beef cattle: are the domestic cattle to produce meat.</p> <p>Calf: as a young female or male bovine up to weaning.</p> <p>Herd: a group of animals that live or are kept together.</p>
Intervention	<p>Disbudding: refers to prevention of horn growth before it has become advanced.</p> <p>Dehorning: the amputation of horns at any stage after their growth of the early budding stage.</p> <p>Castration: is the process of removal, damage, or destruction of the testicles.</p>
Outcome	<p>Animal welfare or animal well-being: involves basic health and functioning, natural living and affective state.</p> <p>Animal pain: is an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or is describable in terms of such damage.</p> <p>Animal stress: biological response elicited when an individual perceives a stressor to its homeostasis.</p> <p>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.</p> <p>Behaviour: farm animal welfare behaviour has been used to assess the response to painful husbandry procedures.</p> <p>Behavioural indicators, measured objectively or subjectively, can provide robust assessment tools for pain with that they are clearly explained and validated.</p> <p>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.</p>

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 <i>et al.</i> 1995	Peer-reviewed	Canada	180 / 12	Amputation dehorning	NA	Cortisol (30 minutes)
Mellor <i>et al.</i> 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 <i>et al.</i> 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	<i>Bos taurus taurus</i>	1 (1)
	<i>Bos taurus indicus</i>	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 <i>et al.</i> 1995	High	High	Low	Cortisol	Unclear	Low	Low
Mellor <i>et al.</i> 2002	Low	Unclear	Low	Cortisol	Unclear	Low	Low
Sinclair 2012	Low	High	Low	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
Sinclair 2012	Low	High	Low	Vocalisation	Unclear	High	Low
				Cortisol	Unclear	Low	Low
Sinclair 2012	Low	High	Low	ADG	Unclear	Low	Low
				Cortisol	Unclear	Low	Low
Sinclair 2012	Low	High	Low	ADG	Unclear	Low	Low
				Vocalisation	Unclear	High	Low
Sinclair 2012	High	High	High	Cortisol	Unclear	Low	Low
				Vocalisation	Unclear	High	Low
Hubber <i>et al.</i> 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

Variable	Assessment	Number of publications (studies)		
		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)
	Yes	1 (3)	3 (6)	1 (3)
Was the statistical analysis described adequately so it can be reproduced?	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)
	Yes	1 (3)	3 (6)	1 (3)

ADG: average daily gain

^AComputer or random number table, *a priori*, stratified random sample, cluster random sample.

^BAuthor(s) report random, but randomization is not described.

^CTaken n samples at interval of x or stratified by certain characteristics.

^DAuthor indicated convenience sampling or sampling was not reported in the paper.

^EClustering was evaluated when repeated measures were reported.

^FAuthor identified confounders and controlled for them in the analysis.

^GConfounders were identified and included/excluded a priori.

^HConfounders were controlled a priori by matching on certain characteristics.

^INo adjustments were made for confounders/effect modifiers, etc., that were identified by the author.

^JConfounders 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

	Publication (studies)	ADG	Studies (trials)	
			Cortisol	Vocalisation
<i>Amputation dehorning</i>				
Pain relief				
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)
<i>Total</i>	<i>3 (6)</i>	<i>3 (15)</i>	<i>6 (31)</i>	<i>3 (10)</i>
<i>Cautery dehorning</i>				
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)
<i>Total</i>	<i>2 (2)</i>	<i>0 (0)</i>	<i>2 (13)</i>	<i>0 (0)</i>

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 ($0.05 \leq P < 0.1$) covariates investigated as potential 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-R²: percentage of the residual variation;

NSAID: non-steroidal anti-inflammatory drug.

^ANumber of studies included in the meta-regression.

^BNumber of trials included in the meta-regression.

^CStandard mean difference of the effect size.

^DThese values represent 95% confidence intervals (CI) for the effect size.

^ESignificance 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 (◆).