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1 **A meta-analysis of cortisol concentration, vocalization, and average daily gain associated**
2 **with castration in beef cattle**

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16 **ABSTRACT**

17 A systematic review and meta-analysis (MA) were performed to summarize all scientific
18 evidence for the effects of castration in male beef cattle on welfare indicators based on
19 cortisol concentration, average daily gain (ADG), and vocalization. We searched five
20 electronic databases, conference proceedings, and experts were contacted electronically. The
21 main inclusion criteria involved completed studies using beef cattle up to one year of age
22 undergoing surgical and non-surgical castration that presented cortisol concentration, ADG,
23 or vocalization as an outcome. A random effect MA was conducted for each indicator
24 separately with the mean of the control and treated groups. A total of 20 publications
25 reporting 26 studies and 162 trials were included in the MA involving 1,814 cattle. Between
26 study heterogeneity was observed when analysing cortisol ($I^2 = 56.7\%$) and ADG ($I^2 =$
27 79.6%). Surgical and non-surgical castration without drug administration compared to
28 uncastrated animals showed no change ($P \geq 0.05$) in cortisol level. Multimodal therapy for
29 pain did not decrease ($P \geq 0.05$) cortisol concentration after 30 min when non-surgical
30 castration was performed. Comparison between surgical castration, with and without
31 anaesthesia, showed a tendency ($P = 0.077$) to decrease cortisol levels after 120 min of
32 intervention. Non-surgical and surgical castration, performed with no pain mitigation,
33 increased and tended to increase the ADG by 0.814 g/d ($P = 0.001$) and by 0.140 g/d (P
34 $=0.091$), respectively, when compared to a non-castrated group. Our MA study demonstrates
35 an inconclusive result to draw recommendations on preferred castration practices to minimize
36 pain in beef cattle.

37 **Keywords:** analgesia; animal welfare; pain

38

39 **Introduction**

40 Castration is a common livestock management procedure throughout the world. The
41 physical procedure is the most common approach used by farmers, although it increases
42 cortisol concentration and changes animal behaviour (Fell et al., 1986; González et al., 2010;
43 Roberts et al., 2015). To counteract this, a hormonal method, i.e. immunocastration, has been
44 proposed as an alternative method for castration (Martí, 2012).

45 The awareness of animal pain caused by routine husbandry practices has become more
46 common, and additional studies have been motivated to determine the role of pain relief
47 (Stafford and Mellor, 2005; Roberts et al., 2015). Currently, understanding the effects of
48 castration methods and their relationship with pain management have been discussed in
49 narrative reviews, highlighting that the castration cause pain, but determining the need for
50 analgesia, as well as the dose, route, duration and frequency of drug administration in cattle
51 are still unclear (Stafford and Mellor, 2005; Coetzee, 2011). Additionally, due the difficulties
52 and the variability in field research, the integration of findings from many studies, using
53 systematic review (SR) and meta-analysis (MA) can provide an equally unbiased estimate of
54 the treatment effect, with an increase in the precision of this estimate (Egger et al., 2001;
55 Borenstein et al., 2009).

56 Lean et al. (2009) reported that a rigorously conducted MA could provide new insights into
57 animal well-being. We conducted a SR-MA to test the hypothesis that specific methods of
58 castration and pain relief strategies can be used to prevent or minimize the adverse effects on
59 beef cattle welfare. The purpose of this study was to identify, evaluate, critically appraise, and
60 synthesize the available literature reports on how the castration procedures affect beef cattle
61 welfare using a SR-MA approach.

62

63 **Material and methods**

64 *Research question and protocols*

65 This study identified the effects of castration procedure on beef cattle welfare by
66 measuring cortisol levels, average daily gain (ADG), or vocalization.

67 The literature search strategy was defined based on the main concepts in terms of PICO:
68 population (P), intervention (I), comparator (C), and outcome (O). The population studied was
69 exclusively beef cattle up to 12 months of age (calf or yearling), since the experience of
70 intense pain soon after birth may “programme” the animal’s subsequent sensitivity to pain
71 challenges (Viñuela-Fernández et al., 2007). Moreover, it may not be possible to castrate
72 young calves in extensive beef production systems where calves may not be mustered until
73 weaning (Stafford, 2007). The intervention of interest was castration, dehorning, or disbudding.
74 The present study only shows findings on castration intervention; however, the literature
75 search was conducted to also include other two procedures, dehorning and disbudding, as
76 presented in the flow diagram (Fig. 1). Similar groups of animals undergoing the same
77 procedure, with or without intervention, were considered as comparison groups. We did not
78 exclude publications based on the type of comparison used. Outcomes of interest were
79 vocalization, cortisol, and ADG (see Table A.1).

80 An *a priori* protocol was developed, and each screening tool for this study was adapted
81 from previously available forms (Mederos et al., 2012), and pre-tested before implementation.

82

83 *Search methods for identification of studies*

84 A list of final search terms and algorithms was summarized by population, outcome, and
85 intervention components as follows: (bovine OR “beef cattle” OR cal* OR herd) AND
86 (disbud* OR dehorn* OR castration) AND (“animal wel*” OR “animal pain” OR “animal
87 stress” OR cortisol OR behavio* OR vocali*). This search strategy also retrieved relevant
88 studies of animal performance evaluation as the outcome. Therefore, ADG was not included
89 to avoid an overload of non-relevant citations.

90 A systematic literature search was conducted on May 2013 and updated on May 2015
91 using five electronic databases – CAB Abstracts (Thomson Reuters, 1910–2015), ISI Web of
92 Science (Thomson Reuters, 1900–2015), PubMed (MEDLINE, 1940–2015), Agricola
93 (EBSCO, 1970–2015) and Scopus (Elsevier, 1960–2015). In addition, the following
94 proceedings were searched for references: ADSA-ASAS Joint Annual Meeting (from 2001 to
95 2014) and International Society for Applied Ethology, ISAE (from 2001 to 2014).
96 Researchers in animal welfare were contacted electronically to request unpublished data.
97 Reference search validation was performed by searching the reference lists from four recent
98 literature reviews (Bretschneider, 2005; Weary et al., 2006; Coetzee, 2011; Schwartzkopf-
99 Genswein et al., 2012). All citations were imported into the reference manager RefWorks
100 (RefWorks–COS, USA) and duplicate citations were removed manually.

101

102 *Study selection criteria and relevance screening*

103 Five reviewers contributed to the study and were trained for the relevance screening step
104 using 30 abstracts. With that, we sought to identify potentially relevant studies, for
105 determining relevance of the studies identified by search strategy, for critically appraising the
106 studies, and for analysing variation among studies (Higgins and Green, 2011). The citation
107 was considered relevant when investigating primary research; animal welfare in beef cattle;
108 castration, dehorning, or disbudding as interventions; and measuring cortisol, vocalization, or
109 ADG as welfare indicators. The included study designs were randomized and non-randomized
110 clinical trials, cohort studies, and case-controls. At this stage, no limits were applied for
111 language or publication year.

112 Finally, all identified citations were independently assessed for relevance by two
113 independent reviewers using the titles and abstracts (when available). Data conflicts were
114 determined through discussion, and an expert opinion was requested when agreement was not

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

117

118 *Methodological assessment and data collection process*

119 Data extraction (DE) forms were adapted from previous work. The first author was
120 responsible for data extraction from the eligible studies. Publications reporting more than one
121 study design were duplicated and extracted as separate studies.

122 Before risk of bias assessment and DE were performed, the relevance of papers selected
123 through abstract screening was confirmed using the full papers based on language (English,
124 Spanish, Portuguese, or Italian); appropriate control group; sufficiently detailed results to
125 conduct the DE and to extract quantitative data for MA. At this stage, primary research was
126 restricted to publications in the languages in which the research team members were fluent,
127 since translation of published articles in other languages was precluded due to financial
128 constraints.

129 Information extracted from each study was divided into study population, intervention,
130 outcome measurements, and result data. Manuscript-level information included the journal
131 name, the author(s) name(s), the year of publication, and the original language.

132

133 *Considerations for data collection and manipulation*

134 For each outcome, we attempted to extract the mean, standard deviation (SD), or any
135 available measure of dispersion, measurement unit, *P*-value, and the number of animals in the
136 control and treatment groups. Cortisol and ADG data were converted to nmol/L and g/d,
137 respectively. The included studies in our database evaluated the vocalization in the same
138 scale, 0 to 3 (0= no vocalization; 1= snorting or grunting; 2= momentary vocalization; and 3=
139 continuous vocalization during and immediately after testicular manipulation). These

140 summary measures were entered into an electronic spreadsheet, and a dataset was built
141 containing the results of controlled studies and outcomes of interest: cortisol (baseline, 20 or
142 30 or 40min, and 120min), ADG (during observation period), or vocalization scores (during
143 the procedure). The early stages of pain responses, as well as the long-term pain, both induced
144 by castration, have been assessed extensively using cortisol level (Mellor et al., 2000; Thüer
145 et al., 2007; González et al., 2010).

146 For further analysis, the castration methods were stratified into three groups: 1) surgical
147 castration including Newberry knife, knife, and scalpel blade (plus emasculator or Henderson
148 castrating tool¹); 2) non-surgical castration including elastrator rings, band, Callicrate bander²,
149 Burdizzo emasculator (plus elastrator ring), and immunocastration; and 3) surgical vs. non-
150 surgical castration methods (comparison between non-surgical and surgical castration). The
151 control group may be uncastrated (Group 1 and 2) or surgical (Group 1) and non-surgical
152 (Groups 2) castration, and the treated group were always submitted to surgical (Group 1) or
153 non-surgical (Group 2) castration. When the comparison was between two castrated groups,
154 the intention was to compare different techniques of surgical (Group 1) and non-surgical
155 (Group 2) castration. In addition, relevant pain mitigation was identified as analgesic-sedative
156 (xylazine), anaesthesia (lidocaine, and combination of xylazine and ketamine), anti-
157 inflammatory (dexamethasone, dipyrone, ketoprofen, and meloxicam), and multimodal
158 therapy (combination of xylazine and flunixin or procaine, and lidocaine and dipyrone).

¹ The Henderson castration tool is clamped on each spermatic cord individually and rotated by a cordless drill approximately 20 rotations until the cord is severed.

² The band is applied around the scrotum proximal to the testes. The elastic band is tightened until adequate tension is achieved; a metal grommet is then crimped around the band to hold tension.

159 When the study reported the results in the log-transformed scales, these were transformed
 160 back to the original scale using the formula described by Mederos et al. (2012). A pooled
 161 standard deviation (S_p) was derived from the formula when an overall standard error of the
 162 mean (SEMp) was reported for the control and treatment groups (Ceballos et al., 2009;
 163 Higgins and Green, 2011; Mederos et al., 2012) as follows:

$$164 \quad S_p = SEM_p \times \sqrt{n_p} \quad (1)$$

165 Where n_p is the number of calves in the treatment and control groups.

166 In studies that reported only P -values, an estimate of a common standard deviation was
 167 computed using the t -statistic and assuming the data were normally distributed, using the
 168 formula (Ceballos et al., 2009; Mederos et al., 2012):

$$169 \quad S_p = \frac{(x_2 - x_1)}{t(\alpha/dE) \sqrt{(1/n_2) + (1/n_1)}} \quad (2)$$

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

172 When results were only graphically presented, the corresponding author was contacted by
 173 electronic mail and asked to provide the summary statistics. If no response was obtained or
 174 data were not provided, the mean or measure of dispersion or both were extracted by manual
 175 measurement using a ruler. Finally, as the cortisol data were collected in three different
 176 points, the summary data were retrieved, and the effect size was computed according to
 177 recommended methodological approaches (Borenstein et al., 2009).

178

179 *Quality assessment*

180 We used standardized methods to estimate the risk of bias of the individual studies
 181 included in the MA (Higgins and Green, 2011), with one minor modification. To evaluate the
 182 domain “blinding of outcome assessment”, we considered that the vocalization was at high

183 risk of bias if blinding was not reported and at low risk of bias if blinding was reported
184 (Dzikamunhenga et al., 2014). This is a subjective measure and more prone to poor reliability
185 (Weary et al., 2006). Cortisol concentration and ADG were considered to be at low risk of
186 bias regardless of the presence or absence of blinding. Quality assessment was performed by
187 the first author.

188

189 *Meta-analysis*

190 Studies were included in the quantitative analysis when they reported sufficient data to
191 estimate a mean difference (MD) between control and treatment groups and a 95% confidence
192 interval (CI). Cortisol values were obtained from baseline to 20/30/40 min and up to 120 min
193 was analysed, while for ADG we used data collected during the follow-up period reported by
194 the authors. For cortisol, the term “30 min” will be used as a general descriptor for
195 samples collected at 20/30/40 min, since the data were scarce for independent evaluation in
196 each time. The random effect MA and meta-regressions were conducted given *a priori*
197 assumption of between-study heterogeneity using the DerSimonian and Laird (1986) method.
198 All statistical analyses were performed using statistical package Stata V 14.0 (StataCorp.,
199 Texas, USA).

200 Comparison groups. A separate MA was conducted using various subsets of data
201 consisting of at least two individual studies that investigated similar treatments with the same
202 outcome. As shown by many researchers, the MA comparison groups with small number of
203 trials are possible and the results are reliable (Mederos et al., 2012; Falzon et al., 2014; Lean
204 et al., 2014). Concurrently, each outcome was evaluated separately as a group using
205 stratification by castration method and pain management and a pooled MD and 95% CI were
206 generated. Cochran’s Q (chi-square test of heterogeneity) and I^2 (percentage of total variation
207 across studies that is due to heterogeneity rather than chance) were calculated based on the

208 castration method and outcome. The magnitude of I^2 was interpreted in the order of 25%,
209 50%, and 75% and considered as low, moderate, or high heterogeneity, respectively (Higgins
210 et al., 2003).

211

212 *Publication bias*

213 Publication bias was visually and statistically assessed using a funnel plot, Begg's adjusted
214 rank correlation, and Egger's regression asymmetry tests for each outcome. Bias was
215 considered based on visual plot and whether at least one of the statistical methods was found
216 to be significant ($P < 0.10$). If there was any evidence of publication bias, we used the "trim-
217 and-fill" method to estimate the extent of the bias as suggested by Duval and Tweedie (2000).

218

219 *Meta-regression analysis*

220 Univariable random-effects models were performed to evaluate sources of between-study
221 heterogeneity that may influence the cortisol level and ADG as response of subjects to
222 treatment (Borenstein et al., 2009). The variables explored in the meta-regressions were (1)
223 randomization (no or yes), (2) cluster control (no, yes, or not applicable), (3) confounders
224 identified and controlled (no, yes, or not applicable), (4) manuscript publication year, (5)
225 peer-reviewed (no or yes), (6) continent (North America, South America, Europe, Asia, or
226 Oceania), (7) cattle group (*Bos taurus taurus*, *Bos taurus indicus*, hybrid/mixed, or not
227 reported), (8) who performed the intervention (not reported, farm staff, or veterinarian), (9)
228 application of medicine for pain relief (no or yes), (10) type of medicine (not applicable,
229 analgesic-sedative, anaesthesia, anti-inflammatory, or multimodal therapy), (11) method of
230 castration (surgical, non-surgical, or surgical vs. non-surgical castration methods), (12) cattle
231 age (days), (13) intervention follow-up (i.e., it is the sum of the adaptation and the
232 experimental periods), and (14) sample size. As explained above, univariable analysis was

233 performed due to the small number of observations available for each outcome of interest that
234 precluded the development of multivariable analysis.

235

236 *Cumulative meta-analysis and influential studies*

237 A cumulative MA was conducted to evaluate the pooled estimate of the treatment effect
238 each time that a new potential study was published. Those analyses are most often used to
239 display the pattern of the evidence over time (Borenstein et al., 2009). Sensitivity analyses
240 were performed to determine whether certain studies had a substantial effect on the MD by
241 manually replacing and removing one study at a time and evaluating whether the effect had
242 changed by $\pm 30\%$.

243

244 **Results**

245 *Study selection*

246 Our search identified 1,267 citations, from which 102 full-text publications were assessed
247 for eligibility, and 69 were excluded after methodological soundness and data extraction (Fig.
248 1). Of the remaining, 9 publications did not have enough data to perform the quantitative
249 analysis (see Table A.2), and 20 reports on castration were included in the SR-MA (Table 1).

250 Numerical data were obtained from two of 20 contacted authors who presented their results
251 graphically or without sufficient data (one from the USA and one from Uruguay). The
252 treatment groups evaluated in this study were: surgical castration ($n = 19$ studies), non-
253 surgical castration ($n = 17$), and surgical vs. non-surgical castration methods ($n = 14$).
254 Relevant pain mitigation included: two studies analysing analgesic-sedative, seven evaluating
255 anaesthesia, six evaluating anti-inflammatory and four evaluating multimodal therapy. The
256 total number and the average age (days) of cattle included in this MA were, respectively, 402
257 and 134 for cortisol concentration; 1,648 and 214 for ADG; and 32 and 150 for vocalization.

258 In total, 18 publications¹ were included in this SR-MA that comprised 23 studies and 156
259 unique treatment comparisons. The results of the main characteristics of the included studies
260 are presented in Table A.3.

261

262 *Risk of bias*

263 None of the studies provided sufficient details about the blinding of personnel and the risk
264 of performance bias was unclear. The risk of detection bias was considered relevant only for
265 vocalization, and none of the studies used to blind outcome assessor from knowledge of
266 which intervention a participant received, leading to high risk of detection bias. The approach
267 to describe the completeness of outcome data for each main outcome showed a high risk of
268 bias in two studies that evaluated cortisol concentration (Petherick et al., 2012). Both studies
269 showed missing outcome data likely to be related to true outcome, with either imbalance in
270 numbers or reasons for missing data across the intervention group. Several studies failed to
271 give enough detail to assess the potential risk of bias as presented in Table A.4 and Table A.5.

272

273 *Meta-analysis*

274 One hundred sixty three trials from 26 studies were included to perform MA on cortisol
275 concentration and ADG data. The vocalization score was the least investigated outcome, and
276 data were presented in a manner that was not usable in the quantitative MA. There were no
277 exclusions due to lack of randomization procedures or lack of adjusting for clustering and
278 confounders. The number of publications, studies, trials, and type of outcome measurements
279 available for the statistical analyses are presented in Table 2.

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

280 *Effect of castration on cortisol concentration.* The variation in the overall cortisol mean
281 difference attributable to the heterogeneity was high ($I^2 = 56.7\%$).

282 Non-surgical castration: Eight studies ($n = 20$ trials) which evaluated non-surgical
283 castration were included and when no stratification by control group or by the type of pain
284 management were performed, the overall MD was 0.108 nmol/L (95% CI: -0.305, 0.522) with
285 high between study heterogeneity ($I^2 = 80.2\%$; $P < 0.001$). A stratified analysis on trials
286 considered castrated animals without drug administration, at 30 min and 120, revealed no
287 significant effect on cortisol concentration and high between study heterogeneity when
288 compared with non-castrated animals (Table 3). The multimodal therapy yielded a non-
289 significant decrease in cortisol concentration 30 min after procedure with a moderate between
290 study heterogeneity ($n = 2$ trials; $I^2 = 36.2\%$).

291 Surgical castration: Combining data from the eight studies ($n = 30$ trials) that evaluated
292 surgical castration presented cortisol MD of 0.122 nmol/L (95% CI: -0.104, 0.349) with
293 moderate between study heterogeneity ($I^2 = 28.2\%$; $P = 0.077$). In the stratified analysis, cattle
294 submitted to surgical castration without pain mitigation compared to uncastrated did not show
295 an effect on cortisol concentration, at 30 min and at 120 min, with no between study
296 heterogeneity and moderate between study heterogeneity, respectively (Fig. 2). Studies where
297 anaesthesia was used to perform castration did not affect the cortisol level at 120 min in
298 comparison to surgical procedure without drug administration with no between study
299 heterogeneity (Table 3).

300 Non-surgical vs. Surgical castration methods: There was no consistent evidence of an
301 overall effect on the cortisol concentration ($MD = 0.080$ nmol/L; 95% CI: -0.153, 0.314) ($n =$
302 17 trials) with low between study heterogeneity ($I^2 = 1.3\%$). Regardless of the time of cortisol
303 measurement, 30 or 120 min, the stratified analyses showed no and low between study

304 heterogeneity, respectively, and no strong evidence on difference in cortisol level when
305 castration was performed with no pain mitigation in both groups (Table 3).

306 *Effect of castration on ADG.* There was high between studies heterogeneity ($I^2 = 79.6\%$)
307 for the included studies reporting ADG data.

308 Non-surgical castration: Pooled results from 13 studies ($n = 27$ trials) evaluating non-
309 surgical intervention showed an increase on ADG by 0.411 g/d (95% CI: 0.009, 0.812; $P =$
310 0.045) with high between study heterogeneity ($I^2 = 90.4\%$). Results from the stratified
311 analysis presented a higher performance for non-surgical castration without drug
312 administration when compared to the non-castrated group, with high between study
313 heterogeneity. The use of anaesthesia and multimodal therapy had no effect on ADG when
314 compared to uncastrated cattle, with high between study heterogeneity (Table 4).

315 Surgical castration: Pooled analyses across all 14 studies ($n = 44$ trials) that evaluated
316 surgical castration showed no significant difference on ADG ($MD = 0.133$; 95% CI: -0.040,
317 0.306), with high between study heterogeneity ($I^2 = 61.3\%$). Results from the stratified
318 analysis on surgical castration with no pain mitigation reported a tendency to increase the
319 ADG compared to uncastrated animals, with moderate between study heterogeneity. No
320 differences were found in ADG when castration was performed with anaesthesia, anti-
321 inflammatory, or multi-modal therapy (Table 4).

322 Non-surgical vs. Surgical castration: The comparison between non-surgical and surgical
323 castration was reported in eleven studies ($n = 24$ trials). We observed no difference ($MD = -$
324 0.033; 95% CI: -0.293, 0.228) with high between study heterogeneity ($I^2 = 56.8\%$). Non-
325 significant effect and moderate between study heterogeneity was found when both
326 intervention, surgical and non-surgical castration, were performed without drug
327 administration. In addition, the between study heterogeneity was high when anaesthesia, anti-
328 inflammatory or multimodal therapy were used in the surgical group (Table 4).

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Publication bias

The included studies in our MA are highly heterogeneous; therefore, the results should be carefully interpreted. There was some evidence of publication bias in studies measuring cortisol concentration, since the Begg's test was marginally significant and the random-effects "trim-and-fill" indicated that an additional 13 trials would have been necessary to remove this apparent publication bias (or other small-study effects) (Fig. 3). A symmetrical funnel plot and the statistical Egger's and Begg's tests suggested that publication bias was not likely to be present when evaluating ADG as an outcome.

Meta-regression analysis

Meta-regression results on cortisol concentration. Twelve studies ($n = 67$ trials) were submitted to univariate meta-regression. However, none of them contributed to explain the between study variation. Two of 14 (control of confounders, and peer-reviewed) were significantly associated with the trial effect size. Univariable meta-regression indicated that studies reporting that controlling for confounders had a predicted MD in cortisol level of 0.50 nmol/L lower than studies that did not report control for confounders ($P = 0.045$). Meta-regression results also suggested that studies published in a non peer-reviewed journal (including conference proceedings, thesis, and government or research station report) had a marginally lower predicted value for MD (-0.34 nmol/L; $P = 0.054$) compared to studies published in indexed and scientific journals.

Meta-regression results on ADG. The univariable meta-regression was conducted on 20 studies ($n = 96$ trials). None of the variables showed a significant association with the outcome of interest.

354 *Cumulative meta-analysis and influential studies*

355 In the cumulative meta-analysis for cortisol concentration, there was clear evidence of
356 change in the estimated point of the pooled treatments MD, from negative ($MD = -0.012$) to
357 positive ($MD = 0.114$), using collected data from 2009 until 2014. The sensitivity analysis
358 showed that removing two studies (Coetzee et al., 2010; del Campo et al., 2014) decreased
359 and increased the MD from 0.114 nmol/L to 0.069 nmol/L and 0.161 nmol/L, respectively.

360 No evidence for a chronological tendency was found for the ADG outcome. The pooled
361 estimate for the effects of castration on ADG showed a reduction from 0.176 g/d to 0.114 g/d
362 ($P = 0.134$; 95% CI -0.035, 0.263) by removing only one study (Whitlock et al., 2013).

363

364 **Discussion**

365 There is a clear consensus about the moral and ethical treatment of animals undergoing
366 painful procedures. For cattle farmers castration of their animals is an unpleasant but
367 necessary husbandry procedure, that improves beef quality with increased marbling and
368 tenderness, while for the general public it is considered an unnecessarily painful procedure
369 (AVMA, 2009; Stafford and Mellor, 2010).

370 Unfortunately, literature is discordant and not conclusive on what recommendation should
371 be transferred to farmers and practitioners. Due to the relevance of this topic for the beef
372 supply chain, i.e. from farmers to consumers, we intended to synthesize the research
373 knowledge available on this topic, using a meta-analytic approach. Despite the large number
374 of studies identified using the methodology described above, studies providing data in a
375 suitable manner to allow for a broad quantitative analysis was lower than expected.

376 Most publications suitable to be included in this MA were published in the 2000s. The
377 development of methods of recognition, assessment, and management of animal pain has
378 increased in the last 15 years. Also, in the 2000s, the proper management of pain in food-

379 producing animals became a matter of increasing public concern and growing interest leading
380 to new legislation worldwide (Weary et al., 2006) regarding the emergence of castration as a
381 painful procedure. Among the 18 publications, only one was non-English and more than half
382 were conducted in North America.

383 Although reporting guidelines for randomized controlled trials already have been published
384 (Sargeant et al., 2005), we detected unsuccessful report data for sample size justification,
385 random sequence generation, and blinding. As presented above, there was a variable risk of
386 bias for the outcomes of the included studies, which is a common feature reported on many of
387 the published meta-analysis on livestock (Falzon et al., 2014; Golder and Lean, 2016).

388

389 *The effects of castration on vocalization*

390 The quantitative synthesis of approaches for measuring vocalization was not suitable for
391 this study. Although vocal response is potentially a more revealing source of information
392 about animals' experience than other pain indicators, only counting general vocalizations rates
393 without specifying their types is not sufficient for welfare assessment (Watts and Stookey,
394 2000; Manteuffel et al., 2004). Hence, other pain indicators have been used to quantify
395 changes in animal behaviour following castration, i.e. escape behaviour, struggle, locomotion
396 activity, lying time, kicks, chute behaviour, and feeding behaviour (Fell et al., 1986; González
397 et al., 2010; Coetzee et al., 2012; Pieler et al., 2013; Petherick, 2011; del Campo et al., 2014;
398 Moya et al., 2014).

399 In our MA, the potential for detection bias was high and the obvious problem is the
400 inherent subjectivity. Training independent observers using specific criteria and, moreover,
401 the use of automated measures of animal behaviour, can improve the scientific value of vocal
402 response, mainly in welfare assessment (Watts and Stookey, 2000; Manteuffel et al., 2004;
403 Viñuela-Fernandez et al., 2011).

404

405 *The effect of castration on cortisol concentration*

406 Acute pain is a response to an established inflammatory and metabolic process that
407 activates the hypothalamic-pituitary-adrenal axis (Mellor et al., 2000). Therefore, changes in
408 cortisol concentration appear to be particularly useful evaluating pain assessment, despite
409 monitoring value being limited by the difficulty of measuring the system's reaction, as well as
410 the inter-animal variations to the stress response (Moberg, 2000; Möstl and Palme, 2002).
411 Variations in response may also be due to differences in the way in which a castration
412 technique is conducted by different operators (Coetzee, 2013). Thus, this variability decreases
413 our capacity to detect differences among groups, and a greater numbers of animals are
414 required for experimental models (Mellor et al., 2000). Our data showed a great variability in
415 a small sample size (mean = 33.5; minimum = 10; maximum = 60) in the included SR-MA
416 studies.

417 Cortisol response has been widely used to assess well-being in farm animals, providing an
418 indication of the overall noxiousness of the experience. However, comparisons between
419 manuscripts can be difficult due to the previous experience, ability to learn adaptative,
420 concurrent stressors, circadian rhythm, differences in collection sample methods performed
421 by a different operator, pharmacokinetic model, delay between the time of pain relief
422 administration and the onset of analgesic activity, and analytical methodology (Mellor et al.,
423 2000; Möstl and Palme, 2002; Coetzee et al., 2010, 2011). The pharmacokinetics and
424 pharmacodynamics of the main drugs used for sedation, anaesthesia, or analgesia affect the
425 pain management in cattle (Smith, 2013). Studies to evaluate possible circadian rhythm in
426 cattle showed controversial results, as reported by a diurnal ultradian rhythm and a very weak
427 circadian rhythm (Lefcourt et al., 1993) or by no diurnal variation in the endogenous cortisol
428 secretion (Hudson et al., 1975). Also, it has been proposed that low cortisol responses may

429 appear in individuals with high pain threshold or when the physiological effect of castration
430 procedure was easily observed (Stafford and Mellor, 2005). However, the reasons why some
431 individuals may have different responses are still unclear.

432 The evidences in our meta-analysis suggest that the surgical and non-surgical procedures
433 without drug administration, when compared to uncastrated animals, did not increase cortisol
434 levels as expected. One probable explanation for this observation is the abrupt change of the
435 cortisol measurement after an animal intervention that may influence its final conclusive
436 interpretation. Nineteen bibliographic references were screened and analysed by
437 Bretschneider (2005), who showed that castration caused a fast and maximum adrenal
438 corticoid secretion 12 min after the surgical procedure. Second, the explanation is that
439 sampling blood from animals using catheters with minimal non-aversive handling did not
440 stress the animals (Schwartzkopf-Genswein et al., 2005). Thus, the majority of included
441 studies analysed cortisol in blood by single invasive collection samples (Petherick, 2012; del
442 Campo et al., 2014). Third, there were no significant differences between non-castrated and
443 castrated animals in physiological parameters (Coetzee et al., 2008; Petherick, 2012; del
444 Campo et al., 2014). Cortisol concentration may be increased in response to the stress of
445 animal handling itself and as an invasive method, then hardly difficult to distinguish between
446 non-threatening stress and distress (Moberg, 2000). As mentioned, the absence of variation in
447 cortisol responses can be affected by animals' internal and external characteristics.

448 Furthermore, a similar pattern of cortisol levels in surgical vs. non-surgical castration was
449 observed in our study, in agreement to Petherick (2012) and del Campo et al. (2014). As
450 concluded by Stafford et al. (2002), all methods cause an immediate and significant rise in
451 cortisol concentration, but the *ceiling effect* of cortisol responses can lead to underestimate the
452 adverse effects of the most invasive treatments (Mellor et al., 2000). On the other hand,
453 researchers found an increase in cortisol concentration in surgical (Fell et al., 1986) or non-

454 surgical castration (Petherick, 2011). Special attention is needed when interpreting individual
455 differences, cattle age, and different castration techniques that could influence the cortisol
456 results (Stafford et al., 2002; del Campo et al., 2014).

457 Despite the multimodal analgesic approach being more effective in mitigating pain
458 associated with castration than a single analgesic agent (Coetzee, 2013), no effect of
459 multimodal therapy in decreasing cortisol concentration during non-surgical castration was
460 observed in the first 30 min. As highlighted by Coetzee (2011), the main challenges to
461 provide an effective analgesia are the delay between the time of drug administration and the
462 onset of analgesia activity, and the route or method of analgesic drug administration. Thus,
463 we should consider the timing of administration (30 and 1 min before start of the procedure)
464 and the drug administration route (epidural + i.v. jugular and i.m.+ local), as well as the
465 control groups (one maintained intact and another submitted to non-surgical castration) and
466 the strategy used for the pain relief (analgesic-sedative and anti-inflammatory or anaesthesia)
467 of the included studies in this MA. Both studies used saliva samples to measure the cortisol
468 concentration which may be ineffective as an indicator of immediate or chronic pain
469 (González et al., 2010; Pieler et al., 2013). Moreover, the optimum balance of analgesic
470 efficacy can be achieved by the combination of anaesthesia with anti-inflammatory (Coetzee,
471 2011, 2013), that could virtually eliminate the cortisol response during the first 8 h, and by
472 inference, the pain and distress (Stafford et al., 2002).

473 With respect to anaesthesia, we found no evidence that this pain strategy reduces cortisol
474 level after 120 min of surgical castration. Lidocaine, a short-acting local anaesthetic, was used
475 in these studies, which is effective for approximately 45-90 min and reduces the acute distress
476 associated with castration (Coetzee, 2013; Stafford and Mellor, 2005;). Studies detected that
477 anaesthesia can attenuate serum cortisol response (del Campo et al., 2014; Stafford et al.,
478 2002), with no difference in the integrated cortisol response during 60-150 min post-

479 castration in comparison to uncastrated animals (Coetzee et al., 2010). A topical anaesthetic
480 spray can be used to reduce pain for up to 24 h as a practical and affordable approach to beef
481 cattle farm management (Lomax and Windsor, 2013).

482 Publication bias in the literature is likely to be reflected in the MA approaches (Borenstein
483 et al., 2009). In this case, visual assessment and adjusted rank correlation test indicated some
484 evidence of the presence of some bias. Funnel-plot asymmetries may also have resulted from
485 clinical heterogeneity among studies (e.g. poor methodological design) (Lean et al., 2009).
486 Inadequate quality of primary research has also been reported to yield larger effects (Egger et
487 al., 2001). Meta-regression analysis suggested that studies from non-peer reviewed studies or
488 those without control of confounders change the cortisol response.

489 The distinct result pattern observed in the cumulative MA, i.e. over time there was clear
490 evidence that cortisol concentration decreased in castrated cattle, which might be related to
491 the public concern about the welfare of farm animals and to the use of pain mitigation
492 strategies. The changes observed in the effect size can be the result of the increase in the
493 interest in pain caused by routine husbandry practices (Stafford and Mellor, 2005), as well as
494 the improvement in study quality.

495 The average effect size changed after the removal of two and one studies, respectively.
496 Coetzee et al. (2010) was the only study showing no clustering or group hierarchy, using a
497 relatively small sample size ($n= 22$ animals), and high precision of the estimate was obtained
498 directly from the graph published. A study performed by del Campo et al. (2014) was
499 conducted in South America (Uruguay) and the animals were the youngest (7 days of age),
500 but the intervention protocol was not described in sufficient detail.

501 The results of this MA complement and extend previous research describing the effect of
502 castration in cortisol levels. However, results described in the literature are discordant, and
503 additional studies are required.

504

505 *The effect of castration on ADG*

506 Production parameters do not reflect the pain experienced by cattle (Stafford and Mellor,
507 2005) at the moment of animal castration. Then, castration technique may not be as important
508 from a growth rate standpoint, but can have negative effects on the feed intake and
509 performance (Molony et al., 1995; Pang et al., 2008; González et al., 2010; Moya et al.,
510 2014), mainly in the intensified production systems. In addition, the lower body weight gain
511 in castrated males was possibly due to the decrease of testosterone (Fisher et al., 2001; Pang
512 et al., 2008). However, assessment of these parameters is critical if research on animal welfare
513 is to be of relevance for livestock producers (Coetzee et al., 2011) and more research is
514 required to determine the relationship between castration and feed intake, growth rate, and
515 feed efficiency (González et al., 2010).

516 The effect of castration in ADG had the largest number of trials for our MA. A single study
517 was responsible for reducing the effect size and provides a non-significant change in ADG
518 after castration (Whitlock et al., 2013). This influential study was published in conference
519 proceedings. The abstract format does not allow precise, informative presentation of the
520 methodology used and we cannot rely on contacting the manuscript authors (Egger et al.,
521 2001), thus meaning that we did not have access to the final data for a more precise analysis.

522 In the univariate meta-regression analysis we explored the influence of the follow-up
523 period on ADG, and the results showed no effect, i.e. timing that the ADG was measured in
524 the included studies did not influence our result. As shown by many researchers, the
525 differences in performance between non-castrated and castrated males cattle are mainly
526 manifested after puberty at an average age of 10 months (Barber and Almquist, 1975; Lunstra
527 et al., 1978) or when testosterone concentration peaked at 15 months of age (Gerrard et al.,
528 1987). Concentrations of serum LH and testosterone increased linearly with advance age

529 around the time of puberty (Lunstra et al., 1978) and, then, this is the period that occur the
530 biggest contribution of testicular tissue on animal growth.

531 Field (1971) concluded after literature review that bulls gained weight 17% faster and were
532 13% more efficient in converting feed in live weight than steers. The decrease in ADG after
533 castration in the first two weeks of age (Pang et al., 2008; Warnock et al., 2012) and the
534 reduction in body growth rate (Knight et al., 2000; Fisher et al., 2001; González et al., 2010)
535 can be attenuated at 28 days (Coetzee et al., 2012), 30 days (Knight et al., 2000) and 42 days
536 post-castration (Warnock et al., 2012).

537 Despite the above reports, we did not find differences in growth performance favouring the
538 non-castrated group in our MA. Inadequate nutrition (Bailey and Hironaka, 1969; Martin et
539 al., 1978), as well as the more aggressive behaviour (Martí, 2012), can prevent bulls from
540 expressing their greater productive potential for weight gain. Animal body weight can also be
541 related to age at the time of intervention, hormonal status of the control group, castration
542 method, feed intake, feeding activity, feeding program, and the level of performance achieved
543 (Pang et al., 2008; González et al., 2010; Martí, 2012; Warnock et al., 2012). Furthermore,
544 relevant information, i.e. feed behaviour and physiology, were not available in our database.
545 Thus, caution in drawing final conclusions is crucial because the live weights in the short
546 period of observation (minimum = 27 days; maximum = 217 days; mean = 87 days) are
547 difficult to quantify, mainly when the difference in ADG between castrated and uncastrated
548 cattle groups is below 1 kg/day.

549 Although del Campo et al. (2014) showed greater ADG for non-surgical than surgical
550 castration, we found no strong evidence when both groups were compared. Therefore, the age
551 at which male calves were banded or surgically castrated did not affect the weight at 217 days
552 (Baker et al., 2000; Bretschneider, 2005), as well as on gain:feed when castration was
553 performed after 7 months of age (Warnock et al., 2012; Reppening et al., 2013). The

554 castration method may not influence the growth rate in the long term, indicating that beef
555 cattle were able to compensate and recover from the castration technique intervention
556 (Warnock et al., 2012; Pieler et al., 2013).

557 In agreement with our results, Newton and O'Connor (2013) showed that there was little
558 evidence of castration effect on ADG regardless of the type of pain management. It may be
559 that the cattle that experienced distress after the application of medication suffer changes in
560 social status that lead to permanent changes in behaviour. It would be interesting to obtain
561 more information on pain mitigation, i.e. the route of administration, period of exposure, and
562 optimum dose (González et al., 2010; Coetzee et al., 2012).

563 The present MA has several limitations. First, the approach in reporting outcomes often
564 limited our ability to summarize the data, as there was incomplete reporting of summary
565 measures. However, an attempt was made by contacting researchers in the field, as suggested
566 by Lean et al. (2009). We had excluded six full-text studies on castration because they were in
567 German, Japanese, and Bulgarian as explained in the methodology section. Finally, in the
568 absence of robust and specific direct and indirect measures associated with pain, the choice of
569 parameters about welfare and its relationship with castration may be challenging for precise
570 analysis.

571

572 **Conclusions**

573 In summary, this is the first SR-MA that summarized the available literature on the effects
574 of castration on cortisol, ADG, and vocalization in beef cattle. There was limited evidence
575 that the use of pain relief mitigated pain responses to castration, as well as which castration
576 method was less painful. That lack of effect might be due to insufficient doses or inadequate
577 duration of action of the drugs used, or due to low capacity of cortisol and ADG to detect pain
578 caused by castration in beef cattle. The challenge in animal science studies is to provide

579 complete and accurate details of the methodology using standardized guidelines available in
580 the published literature.

581

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588

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768 **Table A.1.** Population, intervention, and outcome search term strings used for the final
 769 search.

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 and, then, in the cortisol level.</p> <p>Behaviour: farm animal welfare behaviour has been used to assess the response</p>

of calves, deer and lambs to painful husbandry procedures. Behaviours indicators, measured objectively or subjectively, can provide robust assessment tools for pain whereby they are clearly explained and validated.

Vocalization: vocalization is a good behavioural indicator of pain in farm animals. Hence, researchers are interested in using vocal behavior as a manner of evaluating animal welfare.

770 **Table A.2.** List of relevant publications excluded from the final dataset in the meta-analysis.

Reference	Country	Treatment	Analgesic regimen	Outcome parameter	Reason for exclusion
King et al., 1991	Canada	Surgical and non-surgical	NA	Cortisol and ADG	Number of animals per group not presented
Coetzee et al., 2007	USA	Surgical	Anti-inflammatory	Cortisol	No baseline value
Boesch et al., 2008	Switzerland	Non-surgical	Anaesthesia	Cortisol	Only median was presented
Currah et al., 2009	Canada	Surgical	Anaesthesia and anti-inflammatory	Vocalization	Numerical data not shown
González et al., 2009	Canada	Surgical and non-surgical	Anaesthesia	ADG	Only p-value was presented
Becker et al., 2012	Switzerland	Non-surgical	NA	Cortisol and ADG	Number of animals per group not presented
Brown et al., 2012	USA	Surgical	Anti-inflammatory	ADG	Number of animals per group not presented
Brown et al., 2013	USA	Surgical	Anti-inflammatory	ADG	Only p-value was presented

Daniel et al., 2013	USA	Non-surgical	Anti-inflammatory	ADG	Only p-value was presented
Moya et al., 2014	Canada	Surgical and non-surgical	Anti-inflammatory	Cortisol and ADG	Insufficient data for this study

771 NA: not applicable.

772 **Table A.3.** Descriptive characteristics of 20 publications reporting 26 studies included in the
 773 meta-analysis.

Variable	Description	Categories	Number of publications (studies)
Study design	Type of study design used	Control studies	20 (26)
Peer-reviewed	Type of literature the work was published	Peer-reviewed	16 (17)
		Conference proceedings	1 (1)
		Thesis	1 (1)
		Government or research station report	2 (7)
Treatment	Type of procedure evaluated	Surgical castration	13 (19)
		Non-surgical castration	13 (17)
		Non-surgical vs. Surgical castration	9 (14)
Data published	Year of study publication	1990-2000	2 (2)
		2001-2015	18 (24)
Medicament	It was used any class of medicament?	No	15 (21)
		Yes	11 (15)
Medicament	If was used any medicament	Analgesic-sedative	2 (2)

	to mitigate pain, which class?		
		Anaesthesia	5 (7)
		Anti-inflammatory	4 (6)
		Multimodal therapy	3 (4)
Cattle group	Cattle group in which interventions were evaluated	<i>Bos taurus taurus</i>	9 (11)
		<i>Bos taurus indicus</i>	0 (0)
		Hybrid / Mixed	11 (13)
		Not reported	2 (2)
Who performed	Who performed procedure	Farm staff	5 (6)
		Veterinarian	17 (14)
		Not reported	3 (3)
Outcome assessed	Parameter used to assess pain in calves	ADG	15 (20)
		Cortisol	10 (12)
Sample size	Size of total study population per study	n≤50	13 (16)
		n= 51-100	6 (8)
		n≥101	3 (3)
Continent		North America	12 (13)
		South America	1 (5)
		Europe	3 (3)
		Asia	0 (0)
		Oceania	4 (5)

774 **Table A.4.** Summary of assessment for methodological soundness and reporting of 20
 775 publications reporting 26 studies including in this meta-analysis.

Variable	Assessment	Number of publications (studies)		
		ADG	Cortisol	Vocalization
Was the sample size justified?	Yes	1 (1)	1 (1)	0 (0)
	No	14 (19)	9 (11)	2 (2)
How were calves assigned to treatment groups?	Random ¹	0 (0)	2 (2)	1 (1)
	Reported random ²	10 (11)	4 (4)	1 (1)
	Systematic ³	1 (1)	0 (0)	0 (0)
	Convenience or unreported ⁴	4 (8)	4 (6)	0 (0)
Was the intervention protocol described in sufficient detail to be replicated?	Yes	15 (16)	9 (10)	2 (2)
	No	1 (4)	1 (2)	0 (0)
	Reference paper	0 (0)	0 (0)	0 (0)
Did the author report that blinding was used to evaluate the outcome?	Yes	1 (1)	2 (2)	0 (0)
	No	14 (19)	8 (10)	2 (2)
Based on the study design was	Yes	15	8 (10)	1 (1)

clustering ⁵ accounted for		(20)		
appropriately in the analysis?				
	No	0 (0)	1 (1)	1 (1)
	Not applicable	0 (0)	1 (1)	0 (0)
Were identified confounders		9		
controlled for or tested?	Yes, analysis ⁶	(10)	7 (7)	2 (2)
	Yes, inclusion/exclusion ⁷	3 (3)	2 (3)	0 (0)
	Yes, matching ⁸	0 (0)	0 (0)	0 (0)
	No ⁹	1 (1)	0 (0)	0 (0)
	Not applicable ¹⁰	2 (6)	2 (2)	0 (0)
Was the statistical analysis		15		
described adequately so it can be	Yes	(20)	9 (11)	2 (2)
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)

776 ¹ Computer or random number table, *a priori*, stratified random sample, cluster random
777 sample.

778 ² Author(s) report random, but randomization is not described.

779 ³ "n" samples obtained at x intervals or stratified by certain characteristics.

780 ⁴ Author indicated convenience sampling or sampling was not reported in the paper.

781 ⁵ Clustering was evaluated when repeated measures were reported.

782 ⁶ Author identified confounders and controlled for them in the analysis.

783 ⁷Confounders were identified and included/excluded a priori.

784 ⁸Confounders were controlled a priori by matching on certain characteristics.

785 ⁹ No adjustments were made for confounders/effect modifiers, etc., that were identified by the
786 author.

787 ¹⁰Confounders were not identified by the author or randomization was used to control for

788

789 **Table A.5.** Methodological quality assessment of risk of bias (classified as low, unclear, and high) of the 26 studies included in the meta-analysis
 790 in welfare animals from castrated beef cattle.

Reference	Sequence generation	Allocation concealment	Selective reporting	Outcome measurement	Blinding of personnel	Blinding of outcome assessment	Incomplete outcome data
Fell et al., 1986	Unclear	Unclear	High	Cortisol	Unclear	Low	Low
Faulkner et al., 1992	Low	Unclear	Low	ADG	Unclear	Low	Low
Baker et al., 2000	Low	Unclear	Low	ADG	Unclear	Low	Low
Fischer et al., 2001	Low	Unclear	High	ADG	Unclear	Low	Low
Thüer et al., 2007	Low	Low	Low	Cortisol	Unclear	Low	Low

Coetzee et al., 2008	Low	Low	Unclear	Cortisol	Unclear	Low	Low
				Vocalization	Unclear	High	Low
Pang et al., 2008	Low	Unclear	Low	ADG	Unclear	Low	Low
Coetzee et al., 2010	Low	Low	Unclear	Cortisol	Unclear	Low	Low
				Vocalization	Unclear	High	Low
González et al., 2010	Low	Unclear	High	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
Petherick et al., 2011	High	High	Low	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
Coetzee et al., 2012	Low	Low	Low	ADG	Unclear	Low	Low

Warnock et al., 2012	Low	Unclear	High	ADG	Unclear	Low	Low
Petherick et al., 2012	High	High	Low	Cortisol	Unclear	Low	High
Petherick et al., 2012	High	High	Low	Cortisol	Unclear	Low	High
Martí, 2012	Low	Unclear	High	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
Reppening et al., 2013	High	High	Low	ADG	Unclear	Low	Low
Pieler et al., 2013	Low	Unclear	Low	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
Whitlock et al.,	Low	Unclear	High	ADG	Unclear	Low	Low

2013

del Campo et al., 2014	Unclear	Unclear	High	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	Cortisol	Unclear	Low	Low
				ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	ADG	Unclear	Low	Low
Mintline et al., 2014	Unclear	Unclear	Low	ADG	Low	Low	Low

Moya et al., 2014	Low	Unclear	High	ADG	Unclear	Low	Low
Moya et al., 2014	Low	Unclear	High	ADG	Unclear	Low	Low

791 ADG: average daily gain

792

793

794 **Fig. 1.** Flow diagram indicating the number of abstracts and publications included and
795 excluded in each level. MA: meta-analysis. Adapted from PRISMA guidelines (Moher et al.,
796 2009).

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

799

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

809

810 **Fig. 3.** Funnel plot obtained with the Duval and Tweedie's "trim-and-fill" linear random
811 effect model measuring standard mean difference in cortisol concentration as an outcome. The
812 circles represent the original point estimate for each study (MD) and the circles encased in a
813 square represent the studies that the program imputed ($n = 13$) to create a symmetrical plot.