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CASTRAÇÃO E DESCORNA/AMOCHAMENTO EM BOVINOS DE CORTE: REVISÃO SISTEMÁTICA E META-ANÁLISE

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TESE

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CASTRAÇÃO E DESCORNA/AMOCHAMENTO EM BOVINOS DE CORTE: REVISÃO SISTEMÁTICA E META-ANÁLISE[1]

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RESUMO

Castração, descorna e amochamento são práticas de manejo dolorosas, mas realizadas em bovinos de corte. Resultados experimentais sugerem que a dor pode ser reduzida. Contudo, as evidências são contraditórias. Objetivou-se avaliar o efeito desses três procedimentos em indicadores de bem-estar em bovinos de corte com o uso da revisão sistemática e meta-análise (MA). A pesquisa foi realizada em cinco bases de dados eletrônicas e em anais de congressos, além do contato eletrônico com pesquisadores da área. O principal critério de inclusão foram estudos completos ou não randomizados em bovinos de corte com até um ano de idade, submetidos ou a castração ou a descorna ou ao amochamento, que avaliassem concentração de cortisol e/ou ganho médio diário de peso (GMD) e/ou vocalização. Foi realizada MA para efeitos randomizados para cada procedimento e indicador separadamente com as médias dos grupos controle e tratado. Não foram obtidos dados para a realização da MA sobre amochamento. Um total de 23 estudos, representando 156 ensaios e 1.617 animais foram incluídos na MA para castração; na MA para descorna foram setes estudos, 169 ensaios e 287 animais. Observou-se heterogeneidade entre os estudos para os três indicadores avaliados. Independente do grupo controle, não houve efeito significativo no nível de cortisol em animais castrados sem uso de anestésico ou analgésico. O GMD foi superior para animais castrados de forma cirúrgica (P=0.010; MD=0.231 g/dia) e não cirúrgica (P=0.002; MD=0.883 g/dia) em relação aos não castrados. Nos animais não descornados, a concentração de cortisol foi inferior em 0.767 e 0.680 nmol/L, 30 (P=0.000) e 120 min (P=0.023) pós-intervenção, respectivamente, em relação aos descornados por amputação. A anestesia local reduziu os níveis de cortisol 30 min após a descorna mecânica. Animais não descornados tenderam a vocalizar menos (P=0.081; MD=-0.929) que os descornados. Foi observado viés de publicação (cortisol na castração e GMD na descorna), indicando que estudos com amostras pequenas e não significativos são menos propensos a serem publicados que estudos similares e significativos. Ficou evidente a necessidade de pesquisas sobre estratégias para minimizar o estresse e a dor experimentada pelos bovinos durante e após a castração e a descorna, além da busca por indicadores comportamentais e fisiológicos menos invasivos.

Palavras-chave: bem-estar animal, cortisol, desempenho, dor, vocalização

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CASTRATION AND DEHORNING/DISBUDDING IN BEEF CATTLE: A SYSTEMATIC REVIEW-META-ANALYSIS APPROACH[1]

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ABSTRACT

Castration, dehorning and disbudding are painful practices, although conducted in beef cattle. Despite researches suggest that pain can be reduced, the evidences are not conclusive. We aimed to assess the effects of those three procedures in beef cattle on welfare indicators by systematic review-meta-analysis (MA) approach. We searched on five electronic databases and conference proceedings, as well as we electronically contacted experts. The main inclusion criteria were complete and non-randomized studies using beef cattle until one year of age, undergoing castration or dehorning or disbudding that reported cortisol concentration or average daily weight gain (ADG) or vocalization as the outcome. Random effect MA was conducted for each procedure and indicator separately with the mean of control and treated group. There are no data available to analyse dibudding using MA procedure. A total 23 studies, with 156 trials and 1,617 animals was included in the MA for castration; MA for dehorning included seven studies, 69 trials and 287 animals. Significant heterogeneity between studies was observed for all evaluated outcome. Regardless the control group, there was no significant changes on cortisol when castration was performed without anaesthetic or analgesic cover. The ADG showed an increase of 0.883 g/day and 0.231 g/day for non-surgical (P=0.002) and surgical castration (P=0.010), respectively, compared a non-castrated group. Non-dehorned animals showed lower cortisol concentration of 0.767 and 0.680 nmol/L, 30 (P=0.000) and 120 (P=0.023) min after intervention, respectively, when compared with amputation dehorning group. Local anaesthesia reduced increases in cortisol concentration 30 min after dehorned by amputation. Non-dehorned cattle had a marginal significant (P=0.081; MD=-0.929) decrease in the number of vocalization than in dehorned by amputation. Publication bias was observed (cortisol in castration analysis; ADG in dehorning analysis), indicating that small size studies reporting non-significant results were less likely to be published than similar studies that found a significant effect. Researches about effective strategies to alleviate the stress and pain experienced by castrated and dehorned cattle are necessary, as well as validate less invasive physiological measures and behavioural indicators.

Keywords: animal welfare, cortisol, pain, performance, vocalization

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SUMÁRIO

Página

RELAÇÃO DE TABELAS

Página

RELAÇÃO DE FIGURAS

Página

RELAÇÃO DE ABREVIATURAS E SÍMBOLOS

AINE	Anti-inflamatório não-esteroidal
BEA	Bem-estar animal
g/dia	Gramas por dia
GMD	Ganho médio diário de peso
h	Hora(s)
I^2	Heterogeneidade entre estudos
nmol/L	Nanomol por litro
MA	Meta-análise
MD	Diferença entre médias (*mean difference*)
min.	Minuto(s)
RS	Revisão sistemática

CAPÍTULO I

“You’ll find that life is still worthwhile, if you just smile”
(Charles Chaplin)

1. INTRODUÇÃO

As previsões evidenciam que o aumento da população, da urbanização e da renda vem desencadeando maior demanda por alimentos de origem animal. Estudos mostram que a produção de alimentos está em crescimento constante, graças a maior eficiência produtiva. Tal eficiência é decorrente dos avanços genéticos, nutricionais e de manejo, os quais, além de proporcionarem benefícios sociais e econômicos, podem acelerar a degradação ambiental e prejudicar o bem-estar dos animais (Paranhos da Costa & Morales, 2011).

Uma dos aspectos importantes do BEA é a minimização da dor. “Livre de dor, lesões e doenças” foi reconhecido pelo Conselho de Bem-Estar na Produção Animal do Reino Unido (*Farm Animal Welfare Committee,* FAWC) como um dos cinco requerimentos básicos para garantir o bem-estar dos animais de produção (FAWC, 2009). Partindo da teoria da seleção natural de Charles Darwin, indivíduos que passam por certos desafios, p. ex. dor, são mais hábeis em permanecer vivos. Apesar desse valor de aprendizagem e de adaptação, a dor também pode comprometer indicadores fisiológicos, comportamentais, neuroendócrinos e de desempenho. A grande dificuldade é mensurá-la, já que são animais presa e não manifestam claramente (leia-se, falam) o que sentem. Conforme Guatteo et al. (2012) essa é, provavelmente, a principal causa do porque da existência e níveis de dor terem sido subestimados no século XX.

Procedimentos de manejo que provocam dor e sofrimento, como descorna, amochamento e castração em bovinos de corte, podem ser interpretados de forma negativa pelas pessoas. Ao longo dos últimos anos, preocupações advindas da sociedade com relação ao tratamento moral e ético dos animais tornaram-se recorrentes. Por isso, a implementação de legislações com o intuito de assegurar o bem-estar dos animais submetidos a procedimentos dolorosos é realidade em diversos países, apesar das dificuldades inerentes às distintas percepções dos produtores – profissionais – consumidores.

Mesmo com a importância do tema, o conhecimento presente na literatura sobre como avaliar e mitigar a dor ainda não é esclarecedor. Por isso, a realização de pesquisas científicas e o desenvolvimento de soluções práticas são fundamentais na busca por indicadores não invasivos e fidedignos e no desenvolvimento de estratégias que maximizem o bem-estar animal.

Visando esclarecer algumas dúvidas, o presente estudo baseou-se na coleta sistematizada de dados com posterior análise estatística, a meta-análise, sobre a castração, a descorna e o amochamento e seus efeitos no bem-estar de bovinos de corte até um ano de idade. Para tanto, inicialmente, no Capítulo I serão abordados aspectos gerais sobre bem-estar e dor em animais de produção, mais especificamente, em bovinos de corte. A seguir, serão apresentadas a hipótese, os objetivos e a metodologia geral. Os resultados obtidos serão abordados nos Capítulos III e IV. Por fim, o Capítulo V traz as considerações finais do trabalho.

2. REVISÃO BIBLIOGRÁFICA

2.1 Bem-estar animal e a sociedade contemporânea

Preocupações acerca do bem-estar dos animais de companhia não são novidades: proprietários, tratadores, criadores e veterinários sempre se preocuparam com suas condições e seus cuidados, assegurando saúde e alimentação adequadas (Keeling et al., 2011). Na produção animal, por outro lado, somente nas últimas décadas é que a preocupação tornou-se visível, consequente da modernização na produção de alimentos, com incrementos na produtividade e na lucratividade (Guatteo et al., 2012).

Ao mesmo tempo, a sociedade, progressivamente, tornou-se mais atenta e mais preocupada com o sofrimento animal (Guatteo et al., 2012). Com isso, consumidores passaram a incorporar nas decisões de compra conceitos de BEA - técnicas de manejo, práticas de criação, condições dos alojamentos e potencial impacto da produção pecuária no ambiente (Bernués et al., 2003a; 2003b; Phillips et al., 2009; Olynk, 2012) -, sendo essa somente parte da solução no desenvolvimento de mercados de produtos *animal friendly* (Ingenbleek & Immink, 2011). Conforme Rollin (2010), tal fato é consequência da relação humano-animal não ser justa, mas de aparente exploração.

Como consequência, a cadeia produtiva busca soluções para atender o consumidor. Enquanto que nos sistema de produção observa-se aumento dos esforços para promover melhorias no manejo e na produção pecuária (Lyles & Calvo-Lorenzo, 2014), no mercado há a proliferação de selos de qualidade e nas legislações, de leis anticrueldade (Kelling et al., 2011; Rollin, 2010).

2.1.1 Um breve histórico do bem-estar animal

O processo de domesticação dos bovinos teve início há mais de nove mil anos, no Oriente Médio e no sudoeste da Ásia, sendo que a vaca doméstica é, provavelmente, originária do que hoje é a Turquia, o Irã e o Iraque (Manteca, 2009). Independente da época, o rebanho bovino foi, é e continuará sendo um importante componente da sociedade, ajudando a espécie humana

no fornecimento de alimento, na confecção têxtil e na indústria farmacêutica; na companhia e na segurança; na pesquisa e na educação; nas atividades religiosas; no esporte e no entretenimento (Matthews & Hemsworth, 2012).

Durante a maior parte da história humana, a sociedade civilizada tem expressado um consenso ético social em relação ao tratamento dos animais, às vezes até simplório (Rollin, 2009). Representações de animais podem ser encontradas ao longo da pré-história, a partir de esculturas, religiões e artes, as quais demonstram a importância dos animais para as culturas primitivas. Essa ética é encontrada em livro sagrados – judaico, cristão e muçulmano - que, ao estimular o sacrifício dos animais, impõe que o mesmo seja feito por uma pessoa escolhida, da forma mais rápida e menos dolorosa.

Filósofos ajudaram a questionar, e a melhor entender, a representatividade dos animais na sociedade. Pitágoras (570-500 a.C.) era um defensor do “animismo”, ou seja, de que os humanos e os animais possuíam o mesmo tipo de alma. Já Aristóteles (384-322 a.C.) mostrava que os animais possuíam naturezas, o que chamou de *telos*, a “bovinidade” da vaca, a “porcinidade” do porco.

Entre os séculos IV e XII, a ideia com relação aos animais oscilou entre dois extremos: foram considerados como tendo origens iguais aos seres humanos a como sendo objetos, desprovidos de alma e sem direito algum. No século XIII, Tomás de Aquino já mostrava uma visão mais antropocêntrica e psicológica: pessoas que abusassem dos animais iriam, inexoravelmente, progredir para o abuso contra os humanos. Contudo, Descartes, no século XVII, considerava que os animais eram como máquinas, de uso livre pelo homem. Durante os séculos XVIII e XIX, apesar da pluralidade de opiniões, os pensadores concordavam em proibir a crueldade deliberada, sádica, maliciosa, não convencional e intencional, provocadoras de sofrimento e de dor nos animais sem um propósito, pela punição na negligência no fornecimento de água ou alimento, itens esses incluídos nas leis anticrueldade das sociedades civilizadas (Rollin, 2010).

Tudo isso mudou com a industrialização da agricultura, principalmente, após a Segunda Guerra Mundial, incitando debates acerca do

BEA. Em decorrência da intensificação, os sistemas de produção animal no mundo passaram a ser questionados (Euclides Filho, 2004; Fraser, 2008a; Philips, 2009; Hötzel & Sneddon, 2013). Por isso, com a publicação de três importantes livros - *Animal Machines*, por Ruth Harrison; *Animal Liberation*, por Peter Singer; e *The Case of Animal Right*, por Tom Regan –, das mudanças sociais e da evolução tecnológica, a preocupação ética com relação aos animais, por parte da sociedade, passou a ser evidente nos últimos 50 anos.

Na segunda metade do XX, foram publicadas as “cinco liberdades” pela FAWC e, além de prevenir o sofrimento, passou-se a pensar em bem-estar animal positivo, conceito conhecido como *Life worth living*. Desde então, o crescimento da ciência do bem-estar animal foi marcante, criando uma nova conjuntura que tem se mostrado decisiva sob a ótica ética e econômica (Woods, 2012).

2.1.2 Conceitos de bem-estar animal

Apesar do estudo científico sobre BEA ter se desenvolvido rapidamente nos últimos 20 anos, ainda é um grande desafio defini-lo. O conceito foi sendo aprimorado ao longo dos anos, mas ainda é complexo, dado ao contínuo avanço no conhecimento e às diferentes visões da funcionalidade animal e às relações humano–animal e animal–ambiente (Mellor et al., 2009; Keeling et al., 2011). Além disso, pesquisadores adotam diferentes valores baseados no BEA – funcionamento biológico, comportamento natural e estado afetivo/emoção – como alternativas científicas para acessar e melhorar o bem-estar dos animais (Fraser, 2008b).

Nos anos de 1980, dois importantes pesquisadores contribuíram para melhor compreendermos o BEA. Donald Broom relata que o bem-estar “é o estado do animal em relação as suas tentativas de se adaptar ao seu ambiente, sendo uma característica própria, não algo que pode ser fornecido a ele” (Broom, 1986). Contudo, foi a partir da publicação de *Animal Suffering* por Marian Dawkins, que os etologistas passaram a aceitar, gradualmente, a importância dos sentimentos nos problemas de bem-estar dos animais (Duncan, 2006). Ou seja, para um animal perceber uma determinada condição,

que consideramos refletir no seu bem-estar, é preciso que esteja vivo, consciente e que seja senciente (Mellor et al., 2009). Conforme Dawkins (2006), a senciência ainda é o maior problema da biologia, mas o real respeito aos animais somente ocorrerá quando, como seres vivos sencientes, nós os percebermos com seus próprios direitos, com seus próprios olhos e opiniões e com suas próprias preferências e aversões.

Devido a essa complexidade, pode-se considerar que BEA engloba três diferentes componentes, sendo que a contemplação de qualquer um dos critérios, sem julgar os outros, não garante um bom nível de bem-estar (Figura 1) (Fraser, 2008b). Com a análise de tais fatores pode-se pressupor o nível de bem-estar do animal, sendo o “bom nível” alcançado quando estados negativos estão ausentes e/ou positivos estão presentes (Mellor et al., 2009).

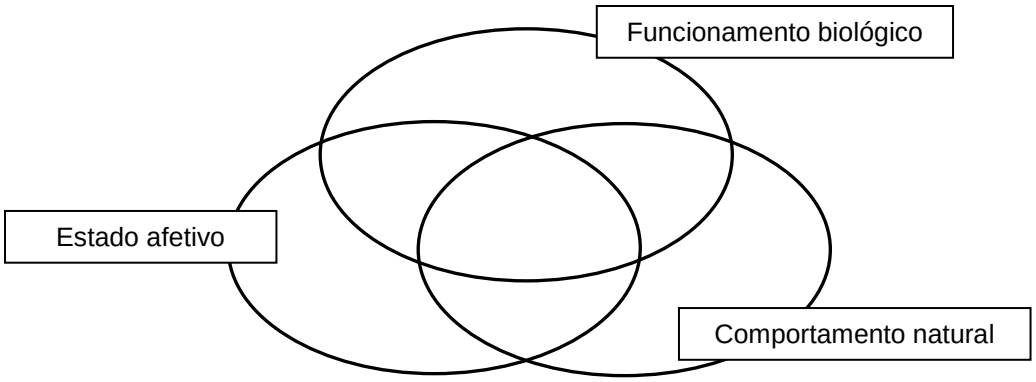

FIGURA 1. Os três elementos do bem-estar animal. Adaptado de Fraser (2008b).

Fatores como crescimento socioeconômico, aumento da preocupação pública em relação ao tratamento recebido pelos animais, maior movimentação das organizações sem fins lucrativos, expansão de evidências na comunidade científica e, por fim, descoberta que os animais são seres sencientes, enfatizam a importância de manter uma condição adequada de bem-estar na produção animal (Poletto & Hötzel, 2012). As bem conhecidas “cinco liberdades” fornecem orientações éticas e práticas para a melhoria do bem-estar de animais de produção (Tabela 1).

TABELA 1. As cinco liberdades do bem-estar animal.

O que?	Como?
Livre de fome e sede	Fácil acesso à água fresca e a uma dieta de acordo com suas necessidades fisiológicas.
Livre de desconforto	Ambiente adequado, incluindo abrigo e área de descanso confortável.
Livre de dor, lesão e doença	Prevenção ou diagnóstico rápido, além de tratamento adequado.
Livre para expressar seu comportamento natural	Espaço adequado, instalações apropriadas e companhia de animais da mesma espécie.
Livre de medo e estresse	Condições e manejo que evitem o sofrimento mental.

Fonte: *Farm Animal Welfare Council* (FAWC, 2009).

Por fim, conforme Fraser (2008b), o bem-estar animal é um conceito que ainda carece de estudos científicos. Entretanto, nossos conhecimentos e a ciência utilizada para acessar o bem-estar são influenciados por valores e ideias consideradas relevantes ou desejáveis para que os animais tenham uma "boa vida".

2.2 Bem-estar na produção animal

2.2.1 Aspectos sociais e éticos

Mudanças na produção agrícola foram relativamente lentas até o século XIX. Foi após o término da Segunda Guerra Mundial que se observaram mudanças na produção agrícola e pecuária (Mellor et al., 2009). Com o crescimento da civilização, a disseminação de novas práticas agrícolas e a modernização tecnológica houve um grande aumento na produção, agora não mais tradicional, mas sim industrial. Com isso, conforme Rollin (2002), a indústria substituiu a criação, além da eficiência e a produtividade terem comprometido o cuidado com os animais.

O modelo baseava-se na intensificação desenfreada e, muitas vezes por desconhecimento, no manejo inadequado, conforme descrito no livro *Animal Machines*, de Ruth Harrison. O sistema não era equilibrado – havia a necessidade constante de *inputs* (fertilizante, herbicida, adubo, ração, forragem, medicamento, vacina) para mantê-lo em funcionamento, do manejo dos resíduos produzidos e do desenvolvimento de drogas e químicos por ele consumido. Considerava-se que os animais tinham uma vida questionável, o

que ainda persiste, com a produção à custa do bem-estar (Rollin, 2010), além de provocar impactos ambientais e desestruturação social (Moreira, 2000; Mazzoleni & Nogueira, 2006).

Infelizmente, a moderna agricultura intensiva industrializada nos permite ignorar a natureza dos animais, e forçar pinos quadrados em furos redondos por meio de soluções tecnológicas (Rollin, 2002). Contudo, com o provável e contínuo crescimento populacional e a crescente preocupação pública, a necessidade de aumentar a produção de alimentos deve, obrigatoriamente, ser pensada na ótica de como minimizar seus efeitos negativos (Mellor et al., 2009). Por isso, princípios éticos devem permear discussões e serem considerados por veterinários, pesquisadores, criadores, enfim, por todos os envolvidos no processo produtivo.

Ao usar animais para nosso propósito, exercemos graus variáveis de controle sobre a qualidade e a duração de suas vidas (Mellor et al., 2009). De acordo com Knierim et al. (2010), isso pode ser feito com cuidados ao bem-estar, o que vai além de motivações intrínsecas ou competitivas – interesse econômico, conveniência, tradição ou conhecimento inadequado –, as quais entram em conflito com os interesses dos animais. A partir disso, questionamentos com relação ao bem-estar dos animais passam a ser consideradas questões de “dever”, de obrigação ética: qual é o nosso dever? E em qual extensão? (Rollin, 2010).

As diferentes visões sobre a consideração moral dos animais não humanos foram revisadas por Palmer & Sandoe (2011). A primeira teoria, o *cartesianismo (contratualismo)*, é pouco influente e questiona o porquê de ser moral e considerar somente os interesses humanos. Essa abordagem foi estendida para o *utilitarismo,* abordagem que pode ser resumida na frase “o bem maior para o maior número (homens ou animais)” e defendida por Jeremy Bentham e Peter Singer. Essa teoria releva somente as consequências quando se tomam decisões éticas, sempre na busca do equilíbrio entre custo e benefício. Foi Bentham, no século XVIII, que argumentou sobre a ideia de que os animais são capazes de sentir dor, sendo essa a primeira abordagem sobre nossas obrigações éticas (Tannenbaum, 1999). A teoria de *respeito à natureza*

dá maior importância à proteção das espécies naturais, à integridade genética e aos processos naturais, sendo os animais valorizados como símbolos da sua espécie. Essas teorias são conhecidas como consequencialistas, uma vez que consideram que os animais podem ser utilizados como um meio para um fim (Cunha, 2012).

Já as teorias de direitos defendem que é um erro usar um indivíduo como mero meio para beneficiar outros, pois isso seria violar um valor inerente enquanto indivíduo (Cunha, 2012). Seres que são sencientes e cognitivos tem diretos a vida, a liberdade e ao tratamento respeitoso, sendo essa a visão dos *direitos morais*. Entretanto, Broom & Fraser (2010) mencionam que a grande dúvida é “o que constitui um direito?”. Uma abordagem mais ampla é a mencionada na *visão relacional*: além de firmar que a relação é mutuamente benéfica, o laço e o compromisso emocional entre humanos e animais são fatores relevantes. Por isso, conforme Cunha (2012), a proposta dos direitos fornece uma base teórica firme para se reivindicar a abolição da instituição do uso de animais, sejam humanos ou não humanos.

Conforme ressaltado por Palmer & Sandoe (2011), apesar das diferentes visões, existem caminhos que permitem conectar as distintas teorias. Essa possibilidade de unificar em uma única teoria normativa, tanto as preocupações utilitaristas quanto as preocupações das teorias morais, foi tentada por Steve Sapontzis, um crítico de Singer (Cunha, 2012).

2.2.2 Estratégias e desafios para aplicação dos princípios do bem-estar animal

A maioria dos envolvidos na produção animal gostaria de ver mudanças a fim de melhorar a vida dos animais. Contudo, com diferentes percepções: o gerente da fazenda busca credibilidade e reputação; o legislador está interessado na execução correta das leis; o pesquisador quer visibilidade e aplicação de seus trabalhos; e o consumidor, que tem tremendo poder para influenciar o BEA, ainda não sabe disso (Whay & Main, 2010). Contudo, é o senso popular do “direito dos animais”, que é guiado pelo respeito à natureza

(*telos*, conforme Aristóteles) dos mesmos (Rollin, 2010), o condutor da criação de princípios na sociedade moderna.

Autoridade em questões relacionadas à saúde animal, a Organização Mundial da Saúde Animal (OIE), passou a inserir padrões de bem-estar em 2005, o qual deve ser considerados pelos seus 180 países membros (outubro de 2015). Contudo, são poucos os padrões estabelecidos se comparados com os diversos acordos intergovernamentais, padrões nacionais, ou até diretrizes das indústrias ou códigos de práticas (Knierim et al., 2010). Por isso, leis e políticas, com objetivo de minimizar a dor e o sofrimento e maximizar o bem-estar dos animais, têm sido adotadas, sendo os países que compõem o Reino Unido e a União Europeia com os regulamentos mais influentes sobre BEA (Matthews & Hemsworth, 2012). Rollin (2009) enfatiza que a sociedade atuou de forma massiva na “grande escrita”, com o compromisso moral de tratar de forma mais adequada os animais.

Nas últimas três décadas essas transformações foram mais impactantes, consequência i) das mudanças na demografia social, ii) dos meios de comunicação, que descobriram que o sofrimento dos animais é de interesse público e iii) da maior consciência da sociedade, a qual passou a se questionar sobre os modelos de produção advindos da Segunda Guerra Mundial (Rollin, 2002). Apesar de negligenciadas em outras regiões, Knierim et al. (2010) enfatizam que, com o tempo, serão reconhecidas, o que já pode ser visível em países da América do Sul e Ásia. Resultados positivos têm sido demonstrados em países da América Latina, onde inúmeras iniciativas (técnicas, legislativas e políticas) estão em desenvolvimento, incluindo programas de treinamento e distribuição de manuais práticos (Paranhos da Costa et al., 2012).

A ciência pode e deve ser uma aliada na geração de informações para auxiliar na tomada de decisões corretas sobre o bem-estar dos animais, apesar de algumas questões éticas ainda não possuírem respostas (Grandin, 2010). Por isso, inúmeros governos e grandes empresas produtoras de carne possuem um conselho consultivo de BEA, composto por leigos e *experts* (Knierim et al., 2010). Tais políticas éticas podem prover perspectivas únicas

sobre pesquisas científicas, adicionando recomendações variadas e específicas (Tannenbaum, 1999).

Na Europa, visando harmonizar as leis dos diversos países que compõem o *Council of Europe*, foi criada uma assembleia exclusiva para questões relacionadas ao BEA, abrangendo transporte internacional de animais, aspectos relativos aos animais de produção, de laboratório e de companhia e ao abate (Knierim et al., 2010). Conselhos mais específicos existem, por exemplo, na Noruega, desde 1993 (*Council on Animal Ethics at the National Institute*), e no Reino Unido desde 1979 (*Farm Animal Welfare Committee,* FAWC).

Em alguns casos, períodos longos de transição previstos na legislação podem facilitar as transformações (Knierim et al., 2010). Por exemplo, entre o lançamento e aplicação da proibição do uso de gaiolas individuais para porcas em gestação e do uso de gaiolas convencionais para poedeiras, na Europa, foram mais de dez anos. De acordo com Poletto & Hötzel (2012), tais mudanças só foram possíveis pela difusão de novas tecnologias, as quais permitiram reduzir o sofrimento desnecessário dos animais.

Já em outros, melhorias no BEA são sinônimos de investimentos (custos), as quais só podem ser alcançadas por outros meios – pelo pagamento adicional de impostos ou por programas de certificação -, alcançando preços maiores de comercialização de forma a sustentar o progresso (Knierim et al., 2010). Pesquisadores confirmaram que a demanda por produtos amigáveis ao bem-estar animal tem aumentado nos últimos anos, mesmo sendo mais caros e menos disponíveis, havendo necessidade do uso de rótulos confiáveis e com credibilidade (Vanhonacker et al., 2010; Kehlbacher et al., 2012). Esses mesmos autores mostraram que 60% dos entrevistados estão dispostos a pagar um preço *premium*, sendo a bonificação variável entre 11 e 18%, o que pode representar um aumento nos gastos mensais com carnes de 26 a 34%.

Enfim, o direcionamento de mudanças para melhorar o BEA só vai ser possível com o envolvimento público – agências governamentais nacional e

internacional – e privado – agentes da indústria de produção de alimentos que trabalham no desenvolvimento de padrões de qualidade (Matthews & Hemsworth, 2012).

2.3 Dor e bem-estar em bovinos de corte

2.3.1 Abordagem e princípios

A Associação Internacional para o Estudo da Dor (*International Association for the Assessment of Pain*, IASP) define a dor como "*uma experiência sensorial ou emocional desagradável associada a um dano tecidual real ou potencial, ou descrita em termos de tal dano*" (Merksey, 1979). Apesar de não existir uma definição padrão aceita pela comunidade científica para os animais (Viñuela-Fernandez et al., 2007), e por acreditar-se que os mesmos possuem um sistema nervoso semelhante ao de seres humanos adultos, qualquer procedimento ou lesão que seja considerado doloroso pelos seres humanos adultos, também o é para os animais, mesmo quando não há evidência patente de comportamento doloroso (Hardie, 2002).

A dor é uma sensação que, por si só, é extremamente aversiva, sendo sua percepção parte do estado do indivíduo (Broom, 1991). Contudo pode ser necessária e benéfica ao sinalizar a presença de algum problema. De acordo com Bateson (1991), a dor, dentre outras características, i) ensina a evitar condições previamente associadas a um estímulo potencialmente perigoso; ii) dá prioridade máxima a fuga ou ao reflexo de retirada consequente de um estímulo potencialmente perigoso, além de evitar condições que tenham sido previamente associadas a tal estímulo; e iii) inibe atividades que poderiam retardar a recuperação de uma doença ou ferimento.

Independente do tipo de procedimento doloroso ao qual o animal é submetido, Tannenbaum (1999) relata que alguns princípios devem ser considerados:

Igualdade: a uma dada quantidade, duração ou severidade de dor, a experiência é a mesma para qualquer espécie, seja humana ou animal;

Justificativa: qualquer um que provoque a dor em um ser que pode vivenciá-la deve mostrar que a dor é necessária e justificável;

Valor: quanto maior a dor experimentada pelo animal, maiores devem ser as justificativas, pois maior é o seu valor;

Mitigação: quando possível, a experiência da dor não deve ser sentida, já que o ato de “não sentir” é a melhor maneira de minimizar a dor.

A prevenção do início da quebra da homeostase, do estímulo nocivo e sua percepção e da ocorrência da cascata dor-estresse-desconforto são de responsabilidade dos profissionais encarregados da saúde animal (Short, 1998; Tannenbaum, 1999; Anderson & Muir, 2005; Viñuela-Fernandez et al., 2007). Entretanto, por sabermos pouco sobre a dor animal e conhecermos pouco os animais (Rollin, 2002), sondar e mensurar a vida emocional são ainda desafios da ciência (Molony & Kent, 1997; Weary et al., 2006). Diante disso, Guatteo et al. (2012) propõem uma nova abordagem para avaliar a dor, conhecida como “3S” (“suprimir, substituir e suavizar”), que está baseada em três pilares - viabilidade (restrições técnicas, logísticas e legais), aceitabilidade (aspectos financeiros e sociais) e utilidade.

2.3.2 A neurobiologia da dor

Antes de elucidar aspectos fisiológicos e patológicos da dor, é preciso introduzir alguns termos (Hellebrekers, 2002b; Muir III, 2009b; Viñuela-Fernandez et al., 2011): i) nocicepção está relacionada com o reconhecimento de sinais no sistema nervoso, os quais se originam em receptores sensoriais (nociceptores) e fornecem informações relacionadas ao dano tecidual, sendo a dor a experiência da nocicepção; ii) neuroplasticidade é a capacidade do sistema nervoso em alterar ou adaptar suas funções bioquímicas e fisiológicas em resposta a estímulos ambientais (exteriores) e biológicos (interiores), os quais são responsáveis pelas sensações dolorosas; iii) antropomorfizar é a atribuição de uma forma ou personalidade humana a um animal, ato moral e eticamente apropriado.

A integração e a inibição de sinais dolorosos ocorrem ao longo de todo o sistema nervoso central (George, 2003). Com isso, a detecção, a transmissão e a geração de respostas fisiológicas e comportamentais são de

responsabilidade de um conglomerado de elementos nervosos (Anderson & Muir, 2005) (Figura 2). Esse aparato de detecção da dor é, entre as espécies, aparentemente de igual sensibilidade, o que pode ser variável é o nível de tolerância (Mellor et al., 2000).

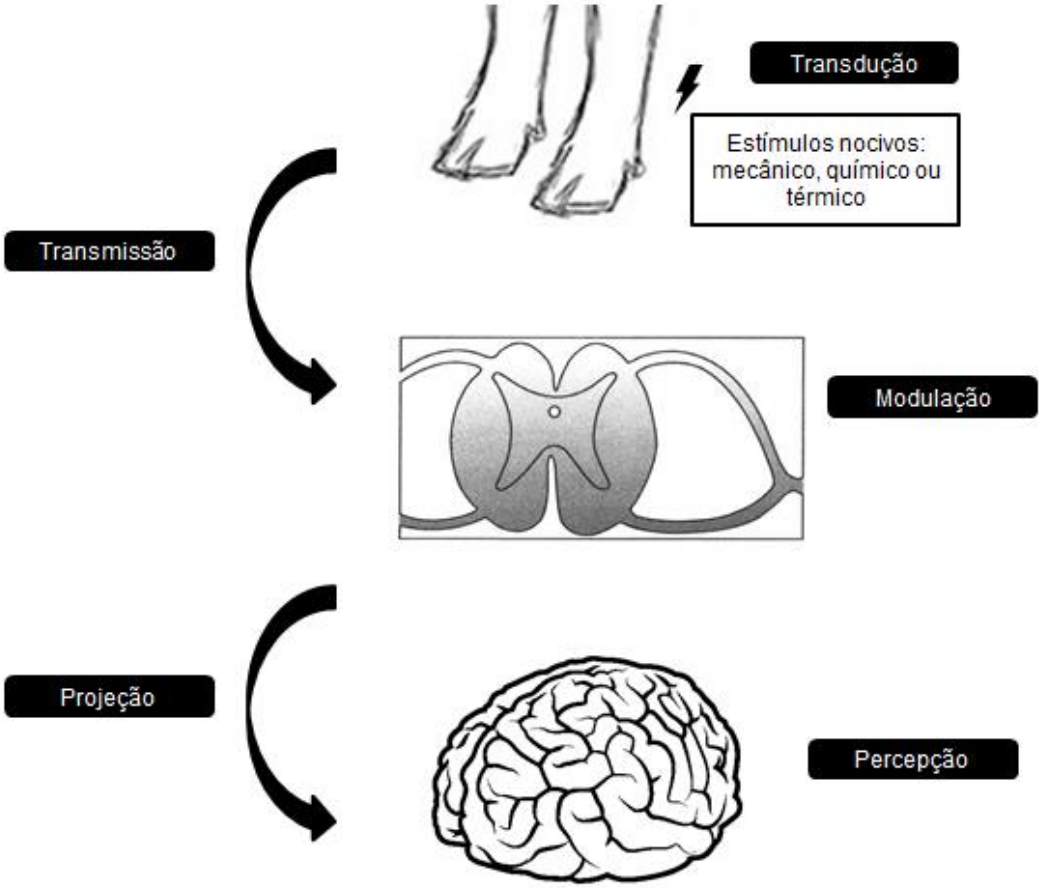

FIGURA 2. Vias envolvidas na interpretação da dor. Estímulos nocivos são transduzidos em sinais elétricos, os quais são transmitidos à medula espinhal, local onde são modulados antes de serem projetados ao cérebro para processamento final e sensibilização. Adaptado de Anderson & Muir (2005) e Muir III (2009b).

A percepção da dor envolve populações de neurônios sensoriais especializados anatômica e fisiologicamente (Viñuela-Fernandez et al., 2011). Nociceptores mecânicos (fibras Aδ), termomecânicos (fibras Aδ e C) ou polimodais (fibras C) são responsáveis por detectar as informações inócuas e nocivas e transformar estímulos ambientais em sinais elétricos (Muir III, 2009b).

Além dos nociceptores das fibras mielinizadas (Aδ), as quais transportam os sinais em alta velocidade, e das não mielinizadas (C), que possuem uma baixa velocidade de condução, existem os nociceptores “dormentes” (fibras Aδ e C). Esses são ativados quando sujeitos a uma severa deformação, tornando-se ativos e capazes de transportar sinais depois de um período de inflamação (Hellebrekers, 2002b; George, 2003; Muir III, 2009a).

A ativação nociceptora, decorrente de um dano tissular, é acompanhada do processo inflamatório no local da lesão e nos tecidos circundantes. Esse fenômeno - sensibilização periférica - é produzido pela liberação de aminas vasoativas e de neuropeptídios, produzindo a hiperalgesia primária. Tais peptídeos estimulam a liberação de mediadores químicos inflamatórios pelos linfócitos, macrófagos e neutrófilos, amplificando a resposta inflamatória e expandido a hipersensibilidade dos tecidos circundantes (hiperalgesia secundária). Essas substâncias juntas produzem uma “sopa de mediadores” que, além de transformar nociceptores de alto limiar em nociceptores de baixo limiar, ativa os nociceptores “dormentes”, amplificando ainda mais resposta de dor (Hellebrekers, 2002b; Klaumann et al., 2008; Manteca, 2009; Muir III, 2009b).

Os sinais elétricos, conhecidos como “potenciais de ação”, são transmitidos ao corno dorsal da medula espinhal, onde são modulados (amplificados ou suprimidos) e é gerado o reflexo de retirada. As informações sensoriais, então, são levadas ao córtex cerebral, o qual as integra, processa e reconhece, produzindo uma resposta integrada (Viñuela-Fernandez et al., 2007; Muir III, 2009b).

A sensibilização central ocorre por uma mudança na excitabilidade dos neurônios na medula espinhal e/ou uma ativação das células da glia da medula espinhal, contribuindo para a hiperalgesia primária (Muir III, 2009b). Conforme Anderson & Muir (2008), a extensão dessa sensibilização leva ao desenvolvimento ou à modificação dos padrões de memória, sendo responsável pelo desconforto e pela agonia causados pelas lesões severas e pelas mudanças comportamentais nos animais.

Mudanças duradouras no percurso da nocicepção – neuroplasticidade - são fundamentais na transição da dor “nociceptiva” para a sua condição “patológica” (Viñuela-Fernandez et al., 2011). A dor é considerada nociceptiva na ausência de lesão tecidual, mas com a ativação de receptores periféricos de dor de alto limiar. Já a dor patológica, que pode ser aguda ou crônica, ocorre na presença ou na ausência de um estímulo ou em resposta a estímulos inócuos, produzindo, frequentemente, uma resposta exagerada e prolongada (Anderson & Muir, 2005; Muir III, 2009b).

2.3.3 O reconhecimento de um estímulo doloroso

Uma das principais dificuldades em reconhecer a dor é diferenciá-la de estresse e desconforto. Resposta ao estresse se refere à totalidade das *reações fisiológicas* – dos ajustes de homeostase às mudanças na fisiologia. Em contraste, a palavra desconforto reconhece o *conteúdo emocional* da experiência nociva, que pode ser de predominância emocional, física ou ambos. Como a dor é de ordem física e emocional, a expressão “desconforto induzido pela dor” reporta a interação desses componentes na experiência nociva (Mellor et al., 2000; 2009).

Durante o século XX, médicos veterinários foram mal preparados, em termos de educação e de ideologia, para tratar da dor animal (Rollin, 2002), acarretando na irrelevância do controle da dor na medicina científica até os dias de hoje (Rollin, 2009). Ademais, o atraso no desenvolvimento de métodos que auxiliem no reconhecimento e no acesso à dor em animais foi decorrente da resistência de alguns pesquisadores em aceitar que os mesmos são capazes de vivenciar e sofrer dor quando sujeitos a ferimentos, lesões, doenças ou outro evento nocivo (Molony & Kent, 1997; Weary et al., 2006).

Possivelmente, a dor nos animais é pior que nos humanos, devido à falta de linguagem e de habilidade de raciocínio sofisticados, fazendo com que não tenham a capacidade de evitar ou antecipar esta sensação (Rollin, 2009). Mais agravante, os animais de produção não são propensos a demonstrar reação a um estímulo doloroso como parte de sua estratégia de evolução, já que são presas e, geralmente, seres estoicos (Currah et al., 2009;

Schwartzkopf-Genswein et al., 2012). No caso dos bovinos, além de sermos incapazes de compreender o que estão realmente vivenciando – sem a existência de uma linguagem comum, não podem contar o que estão sentindo, nem o quão doloroso está sendo um determinado procedimento (Short, 1998; Mellor & Stafford, 1999) – existe variabilidade entre os indivíduos (Moberg, 2000) e influência de fatores relacionados ao grupo social e ao ambiente (Anderson & Muir, 2005).

De acordo com Viñuela-Fernandez et al. (2007), o contraste aparente entre espécies presa e predador pode não ser pela variação no processamento da dor, mas, fundamentalmente, pelas diferenças nas suas respostas ao estresse e ao medo. Com isso, questionamentos de “como ter acesso”, “como mensurar”, “como interpretar” são recorrentes na literatura (Möstl & Palme, 2002).

Atualmente, os biomarcadores da dor são, com frequência, mensurados em amostras biológicas, com o uso de protocolos invasivos (Stubsjoen et al., 2009). Contudo, pesquisas que visam acessar a dor em animais devem utilizar diferentes estratégias - respostas fisiológicas, indicadores comportamentais e variáveis relacionadas à saúde e à produção – a fim de obter uma evidência indireta de um estado mental particular (Tabela 2).

Devido à impossibilidade de se obter medidas diretas de experiências subjetivas, a avaliação da dor animal deve ser realizada por pessoas que conheçam a espécie, a raça e o indivíduo, de maneira a reduzir a tendenciosidade do observador (Molony & Kent, 1997; Short, 1998; Hardie, 2002; Weary et al., 2006; Landa, 2012). Também se deve considerar a variabilidade existente entre espécies e indivíduos, assim como idade, genética, experiência prévia, hierarquia social (Mellor et al., 2000; Moberg, 2000). Finalmente, é importante que os indicadores escolhidos sejam válidos (isto é, devem medir realmente o que se pretende medir), práticos e confiáveis (ou seja, devem proporcionar medidas reprodutíveis) (Manteca, 2009).

TABELA 2. Descrição de índices fisiológicos, comportamentais, de produção e saúde em resposta a um estímulo nocivo.

Indicadores fisiológicos	Indicadores comportamentais	Indicadores relacionados à saúde e produção
Concentração de hormônios sanguíneos Adrenalina, noradrenalina, glicocorticoides, prolactina	Vocalização Lamúrias, rugidos, berros, gritos, mugidos, grunhidos	Doenças Processos inflamatórios (laminite, mastite, artrite), pneumonia bovina (BRD)
Concentração de metabólitos sanguíneos Glicose, ácido lático, ácidos graxos livres, proteínas de fase aguda	Postura Agachar-se, esconder-se, afastar-se, deitar-se (patas estendidas, todas ou algumas patas dobradas), manter-se em pé (com o uso de todas ou algumas patas, com a cabeça contra a parede, pendido para um lado)	Desempenho Peso corporal (ganho de peso), conversão alimentar
Atividade elétrica cerebral	Atividade locomotora Relutância para movimentar-se, andar a passo, esquivar-se, movimentos em círculos, quedas, movimentos repetidos de deita-levanta, inquietação, movimentos com a cauda, lambedura/mordedura no local da lesão	Outros indicadores Quantidade e qualidade das fezes, hemorragia, cicatrização, morbidade, mortalidade
Outras variáveis Nível de leucócitos sanguíneos, resposta imunológica celular e humoral, mudanças nos sistemas cardiovascular e respiratório, tremor muscular, temperatura corporal e retal	Temperamento Dócil, depressivo, quieto, agitado, ansioso, assustado, agressivo	
	Comportamento alimentar Consumo de água e de alimento, tempo de ruminação	

Fonte: Molony & Kent (1997), Mellor et al. (2000), Moberg (2000), Fierheller (2009), Mellor et al. (2009), Muir III (2009b), Broom & Fraser (2010), Stafford & Mellor (2010), Viñuela-Fernandez et al. (2011), Landa (2012), Schwartzkopf-Genswein et al. (2012), Sinclair (2012), Coetzee (2013), Newton & O'Connor (2013).

2.3.3.1 Indicadores fisiológicos

Pela ativação do sistema nervoso simpático ocorrem alterações nos sistemas cardiovascular e respiratório, nas temperaturas corporal e retal e na dilatação pupilar. As informações sensoriais auditivas, visuais e somatossensoriais ativam o eixo hipotálamo-pituitária-adrenal (HPA), o qual aumenta a secreção de hormônios pelo sistema neuroendócrino (cortisol,

catecolaminas, ACTH) (Mellor & Stafford, 1999; Muir III, 2009b; Stafford & Mellor, 2010).

Na maioria dos estudos, o eixo HPA tem sido o primeiro indicador neuroendócrino monitorado, principalmente pelo aumento de glicocorticoides. A concentração de cortisol no sangue é a mais utilizada como indicador de estresse, porém, pelo hormônio possuir ritmo circadiano em muitas espécies e pela simples coleta da amostra (com frequente manejo e contenção dos animais) ser um fator estressante, pode confundir os resultados (Möstl & Palme, 2002; Manteca, 2009; Broom & Fraser, 2010). Com isso, amostras de fezes, de urina, de pelos e de saliva são possíveis alternativas (Möstl & Palme, 2002; Manteca, 2009; Martí, 2012)

A secreção de prolactina, somatotropina e hormônios estimulantes da tireoide mostraram-se igualmente sensitivas aos efeitos do estresse (Moberg, 2000). Tais variáveis, assim como as neuro-humorais, não mensuram a dor *per se*, mas podem auxiliar a compreender e a interpretar o desconforto, que é variável em intensidade, de um estado de mínimo para máximo desconforto (Mellor et al., 2000).

Murata et al. (2004) propuseram a avaliação das proteínas de fase aguda (α_1 ácido glicoproteína, fibrinogênio, haptoglobina), já que sua concentração no sangue é alterada quando os animais são sujeitos a desafios internos e externos, como infecção, inflamação, trauma cirúrgico ou estresse. Aliado a isso, foi comprovado por Coetzee et al. (2008) que a substância P, em conjunto com o cortisol, é uma ferramenta útil para diferenciar desconforto prolongado associado à nocicepção do estresse agudo provocado pelo manejo.

De maneira geral, indicadores fisiológicos, que são métodos indiretos de avaliação da dor, podem ser úteis quando utilizados em conjunto com algum indicador comportamental (Viñuela-Fernandez et al., 2007). Para Grandin (2010), em trabalhos de investigação ainda é relevante acrescentar indicadores produtivos.

2.3.3.2 Respostas comportamentais

A dor, por provocar alterações químicas no cérebro, acarreta em modificações comportamentais (Mellor & Stafford, 1999; Muir III, 2009b). Tipicamente, tais modificações são características facilmente reconhecidas pela sua presença/ausência ou ocorrência em diferentes frequências (Mellor et al., 2000). São indicadores altamente subjetivos (Coetzee, 2013) e variáveis com o tipo de dor e com o estágio de desenvolvimento, mas podem ser robustos se claramente explicados e validados (Millman, 2013).

O comportamento animal se altera em resposta a desafios (Broom, 1991). Conforme Molony & Kent (1997), são quatro as respostas comportamentais que podem ser desencadeadas por um estímulo doloroso: i) resposta automática de proteção, ii) respostas que minimizam a dor e favorecem a cicatrização, iii) àquelas designadas para pedir ajuda ou para interromper a origem da dor e iv) respostas que induzem aprendizagem ou modificam o comportamento animal para evitar a recorrência da experiência.

Como indicadores pode ser avaliada a atividade, o apetite, a atitude, a locomoção, a postura, a vocalização (Mellor & Stafford, 1999; Muir III, 2009b; Stock et al., 2013). Apesar da correlação entre comportamento animal e resposta fisiológica não ser surpresa, visto que ambas as respostas parecem ser controladas, pelo menos em parte, pelo mesmo sistema central neuroendócrino (Rushen, 2000), pesquisas ainda são necessárias para determinar tais relações nos indicadores de dor (Schwartzkopf-Genswein et al., 2012). Ademais, dados comportamentais devem ser interpretados com cuidado, já que nenhum comportamento frequentemente monitorado é específico para um determinado tipo de doença ou de dor (Theurer et al., 2013)

Apesar de o comportamento ser um indicador atrativo, por ser rápido e fácil, a falta de evidências de sua validade pode comprometer sua interpretação (Rushen, 2000). Como os indicadores comportamentais são os mais utilizados para acessar dor em animais de produção, no desenvolvimento de novas técnicas de mensuração deve-se considerar não somente a espécie, a raça e o sexo, mas também o tipo da condição dolorosa (Viñuela-Fernandez et al., 2007).

2.3.3.3 Variáveis relacionadas à saúde e à produção

Durante um estresse prolongado ou severo, o custo biológico é significativo, provocando alterações no funcionamento do organismo e predispondo o animal ao desenvolvimento de patologias (Moberg, 2000). Uma diminuição da produção, assim como danos a integridade física, devem ser consideradas como indicadores de falta de bem-estar (Manteca, 2009).

De acordo com Weary et al. (2006), medidas de produtividade, como alterações no consumo, são mais fáceis de serem mensuradas, porém não refletem o que está acontecendo com animal no momento, mas sim entre sucessivas observações. Já Broom (1991) ressalta que a incidência de doença é de grande importância na avaliação do BEA, uma vez que animais doentes são mais limitados que animais saudáveis.

2.3.3.4 Avanços na avaliação da dor com métodos não invasivos

Pesquisas com métodos não invasivos, complementares aos métodos invasivos, mostraram que a termografia infravermelha, a temperatura ocular e a variabilidade do ritmo cardíaco são úteis para avaliar a atividade do sistema nervoso autônomo somático em animais de produção e para acessar respostas agudas e de mitigação da dor (Stewart et al., 2008; Stubsjoen et al., 2009; Stewart et al., 2010; Coetzee, 2013). Em bovinos minimamente anestesiados, também pode se ter acesso a estímulos nocivos com o uso de eletroencefalograma (Gibson et al., 2007). O uso de acelerômetros é uma ferramenta útil e confiável para prover informações quantitativas da atividade e da postura animal (White et al., 2008).

Indicadores comportamentais podem ser ferramentas robustas para acessar a dor, desde que sejam bem explicados e validados (Millman, 2013). A escala de avaliação numérica (*Numerical Rating Scale, NRS*), apesar de subjetiva, demonstrou boa correspondência com resultados comportamentais, sendo recomendada por Braz et al. (2012) para futuros estudos. Theurer et al. (2013) descreveram, como métodos de monitoramento remoto do comportamento de bovinos, o uso de pedômetros, o controle da duração e

frequência de consumo com o sistema *GrowSafe* ou *Insentec*, controle da movimentação com o uso de GPS (*Global Position Systems)* ou do RTLS (*Real Time Location Systems*). Por fim, Millman (2013) enfatizou técnicas para "questionar" os animais quanto ao nível da dor (teste de sensibilidade térmica, técnica de algometria de pressão) e da experiência dolorosa (teste de evitação condicionada).

Embora as mudanças fisiológicas e comportamentais sejam largamente utilizadas para definir a presença e a intensidade da experiência (Molony & Kent, 1997), a mensuração do BEA é complexa e não existem protocolos padrão. Por isso, indicadores adicionais de bem-estar, além dos indicadores comportamentais e do plasma metabólico, devem ser propostos (Broom, 1991; Molony & Kent, 1997; Viñuela-Fernandez et al., 2007). Coetzee et al. (2008) salientam que identificar e validar indicadores de dor com base na ciência são fundamentais para desenvolver práticas de manejo menos dolorosas e evidenciar protocolos analgésico que minimizem a dor e o estresse, além de maximizar o bem-estar do animal.

"A dor deve ser considerada, até que provem o contrário, como um fenômeno real em animais. O desafio é como mensurá-la."
(Barnett, 1997)

2.3.4 Estratégias farmacológicas para mitigar a dor

Alguns procedimentos dolorosos conduzidos nos animais buscam prover algum tipo de benefício, seja para o animal ou para o criador. Contudo, pesquisas podem fazer com que a sua realização seja repensada: será esse procedimento realmente necessário e justificável? (Weary et al., 2006). Caso a resposta seja afirmativa, qual tratamento paliativo de dor pode ser utilizado? (Manteca, 2009).

Com frequência, mutilações são realizados sem apoio de qualquer tipo analgésico ou anestésico, com base em um argumento de economia de trabalho, duração relativamente curta do procedimento ou estado

sub(desenvolvido) do sistema neurológico dos animais (Hellebrekers, 2002a). Em outros casos, por acreditarem que a dor é de difícil tratamento, ou que o protocolo é impraticável ou inviável economicamente (Viñuela-Fernandez et al., 2011).

Entretanto, com o crescente questionamento da indústria bovina sobre a necessidade de melhorias do BEA, o manejo da dor está ganhando impulso e tornando-se importante na prática (Smith, 2013), já que o uso de medicamento é mais relevante na mitigação da dor que o método utilizado para realizar o procedimento (Phillips et al., 2009). O desconforto pode ser minimizado pela redução do estímulo nocivo durante a contenção, pela escolha de métodos menos dolorosos, pela realização do procedimento em animais jovens e pelo uso de medicamentos (Mellor & Stafford, 1999; Stafford & Mellor, 2010) (vide Seção 2.4). Independente da(s) estratégia(s), conforme Fierheller (2009), deve-se considerar aspectos relacionados à economia, à praticidade e ao bem-estar do animal.

Apesar de poucas drogas serem consideradas capazes de produzir uma analgesia eficiente por períodos prolongados (Muir III, 2009c), a intensidade e a duração do estímulo doloroso são de grande importância na determinação do medicamento a ser utilizado no tratamento da dor (Anderson & Muir, 2005), assim como seu mecanismo de ação (Huxley & Whay, 2006). Apesar do maior número de analgésicos disponíveis para uso veterinário, além do maior interesse e preocupação na mitigação da dor, o quanto disso se converte no controle efetivo da dor ainda é incerto (Viñuela-Fernandez et al., 2011).

2.3.4.1 Uso da anestesia

Anestesia geral é raramente utilizada em ruminantes nas fazendas, pela impossibilidade de aplicá-la em grande número de animais e por não eliminar o desconforto induzido pela dor associada ao processo inflamatório (Mellor & Stafford, 1999; Stafford & Mellor, 2010). Já a anestesia local prévia ao procedimento é amplamente utilizada em animais de produção. Seu mecanismo de ação baseia-se no bloqueio do surgimento e da condução de

impulsos elétricos nos nervos provendo analgesia (Muir III, 2009c). Por isso, são usualmente aplicados próximo aos nervos para bloquear a sensibilização em partes específicas do corpo (Stafford & Mellor, 2010).

Conforme Fierheller (2009) e Stafford & Mellor (2010), a lidocaína é o anestésico local mais utilizado em ruminantes no pré-operatório e mostrou-se efetiva quando utilizada prévia à castração (Fisher et al., 2001; Stafford & Mellor, 2005b; Thüer et al., 2007; Boesch et al., 2008) e à descorna (Graf & Senn, 1999; Sylvester et al., 1998; Mellor et al., 2002; Stilwell et al., 2009). Nos EUA, a sua aplicação epidural ou de bloqueio de nervo em bovinos é permitida na concentração de 2% (Smith, 2013). A absorção é particularmente rápida quando aplicada de forma tópica na mucosa, na forma de gel ou aerossol (Lascelles, 2002; Spinosa et al., 2006), sendo essa uma alternativa segura, viável e prática para melhorar o bem-estar de bovinos submetidos à castração cirúrgica (Lomax & Windsor, 2013) .

2.3.4.2 Anti-inflamatório não-esteroidal (AINE)

Drogas anti-inflamatórias são amplamente utilizadas na prática veterinária, visando aliviar a condição inflamatória (Fitzpatrick et al., 2006), sendo a principal ferramenta no tratamento da dor crônica (Klaumann et al., 2008). Em adição à analgesia, são conhecidos por seus efeitos anti-inflamatórios e antipiréticos. Entretanto, são pouco efetivos na redução do desconforto agudo (Mellor & Stafford, 1999; Coetzee et al., 2012; Coetzee, 2013).

O efeito analgésico é local, na lesão, e central, no cérebro e na espinha dorsal (Fierheller, 2009). A ação do AINE se dá pela inibição de várias isoformas de ciclo-oxigenases (COX-1 e COX-2) e 5-lipo-oxigenase, responsáveis pela produção de prostaglandina e leucotrieno, respectivamente, moléculas com ação pró-inflamatória. A COX-1 é a responsável pelas funções fisiológicas “normais” (constitutivas), estando envolvida na sinalização celular e na manutenção da homeostase, enquanto que a COX-2 é a principal responsável pela superprodução de prostaglandina após lesão ou infecção

(“sistema de intervenção da crise”) (Lascelles, 2002; Spinosa et al., 2006; Muir III, 2009c).

Estudos têm demonstrado os benefícios da analgesia preemptiva (Coetzee et al., 2007; 2012; Currah et al., 2009; Theurer et al., 2012), sendo essa uma das maiores inovações na mitigação da dor em bovinos e ovinos (Stafford & Mellor, 2010). Fármacos inibidores não específicos de COX incluem flunexin-meglumine, cetoprofeno e fenilbutazona. Já os específicos, etodolaco e carprofeno (Andeson & Muir, 2005). Nos EUA, o flunexin-meglumine é o único AINE liberado para uso em bovinos, indicado no controle da pirexia e da inflamação, não no controle da dor. Já o carprofeno é aprovado em diversos países europeus e asiáticos para o controle de processos inflamatórios (Smith, 2013).

Novas gerações de AINE estão sendo continuamente produzidas e comercializadas, p. ex., o meloxicam e o ácido tolfenâmico (Fierheller, 2009). No Reino Unido e em alguns países da Europa o uso de meloxicam, como uma única aplicação intravenosa ou subcutânea, já é permitida, enquanto o ácido tolfenâmico é liberado na União Europeia e no Canadá em bovinos com mastite aguda ou com doenças do trato respiratório (Smith, 2013).

2.3.4.3 Analgésico-sedativos

Os α-2 agonistas adrenérgicos produzem uma analgesia moderada a excelente, com graus moderados a profundos de sedação, pela ativação de uma variedade de subtipos de receptores (α_{2A}, $\alpha_{2B,}$ α_{2C} e α_{2D}) no sistema nervoso central e na periferia. Com isso, há redução na liberação de neurotransmissores relacionados à dor, interferência na transmissão sensorial e analgesia (George, 2003; Muir III, 2009c). A xilazina é utilizada nos bovinos para facilitar o manejo e reduzir a atividade após o procedimento (Lascelles, 2002; Fierheller, 2009) e, mesmo proibida nos EUA, é o sedativo mais utilizado (Smith, 2013).

Opioide é qualquer um, dentre um crescente número de compostos naturais ou sintéticos, que produzem efeitos semelhantes ao da morfina pela ação em receptores opioides, sendo o efeito desejado a produção de analgesia

(Muir III, 2009c). Tem a capacidade de atuar na maioria das células nervosas, promovendo hiperpolarização, inibindo deflagração do potencial de ação e liberação pré-sináptica de neurotransmissores (Spinosa et al., 2006). Apesar de sua ação sedativa e analgésica ter sido amplamente utilizada em animais, não se mostrou muito efetiva nos ruminantes (Lascelles, 2002; Stafford & Mellor, 2010). Na veterinária, a maioria dos opioides utilizados são aqueles seletivos ou parcialmente seletivos aos repectores agonistas μ [OP3], por exemplo, morfina, meperidina e oximorfona (Lascelles, 2002; George, 2003).

Os antagonistas não competitivos de receptores N-metil-$_D$-Asparato (NMDA) previnem o fenômeno *windup*, que é a sensibilização neuronal central, com a consequente sensibilização dos neurônios do corno dorsal. A cetamina, além do efeito analgésico, também auxilia na prevenção das dores aguda severa e crônica (Klaumann et al., 2008; Gaynor, 2009).

2.3.4.4 Fatores que explicam o baixo uso de medicamentos

Estudos relacionados com a dor em animais vêm sendo realizados por quase dois mil anos. Entretanto, somente nas últimas décadas ocorreram avanços reais na compreensão dos mecanismos da dor e no uso terapias efetivas (Lascelles, 2002). Como justificativas para a falta de atenção no controle da dor estão os dogmas ideológicos, as considerações práticas e econômicas, além dos fatores relacionados aos fármacos.

Profissionais dizem que “analgésicos podem mascarar a piora da condição geral dos bovinos” e que “certo nível de dor é útil, já que evita a realização de movimentos excessivos pelos bovinos e acelera a sua recuperação” (Thomsen et al., 2010; Becker et al., 2013). Ademais, Rollin (2002) destaca que se ouve falar que os analgésicos pós-cirúrgicos não são necessários porque os animais “se alimentarão imediatamente”. Apesar desses dogmas, Norring et al. (2014) concluíram que veterinários parecem ter maior empatia com animais que com humanos.

O uso infrequente de fármacos em animais de produção pode ser explicado pelo baixo custo do indivíduo em relação ao custo do tratamento, pela dificuldade logística na aplicação quando o procedimento é realizado em

larga escala, pela necessidade de acompanhamento periódico dos animais, pela regulação governamental, pela falta de comunicação entre produtor e veterinários e pela falta de conhecimento sobre o tema (Viñuela-Fernandes et al., 2007; Newton & O'Connor, 2013). Veterinários entrevistados por Coetzee et al. (2010b) e Thomsen et al. (2010) afirmaram que a falta de aprovação de fármacos e os custos associados ao uso de analgésicos são barreiras que dificultam uso de medicamentos em procedimentos que provocam dor.

Além disso, como habilidade do medicamento em prover analgesia, Schwartzkopf-Genswein et al. (2012) destacam a sua capacidade em mitigar a dor, a sua velocidade da ação, se deve ser usado sozinho ou associado com outro fármaco, o seu modo de aplicação, além da presença de resíduos nos produtos. Dados de farmacocinética estão disponíveis para vários analgésicos e anestésicos em bovinos e, por isso, recomendações sobre o período de carência estão disponíveis (Smith, 2013).

2.3.5 Atitudes à dor bovina

O bem-estar tem assumido importância crescente na produção animal e nos debates acerca de consumo (Vanhonacker et al., 2008). Ademais, os atos de provocar e aliviar a dor são constantemente citados como preocupações-chave da sociedade (Millman, 2013). Com frequência, pesquisas que exploram esses temas são conduzidas na Europa (Vanhonacker et al., 2008; 2010; Kehlbacher et al., 2012).

Vanhonacker et al. (2008), ao compararem a visão do cidadão e do produtor sobre o bem-estar dos animais de produção, na região de Flandres, identificaram similaridades, apesar de maiores escores absolutos de importância serem obtidos dos cidadãos. Além disso, elucidaram discordâncias em alguns tópicos, inclusive nas questões relativas à dor e ao estresse provocados pela intervenção humana. Entretanto, conforme Phillips et al. (2009), outros participantes da indústria da carne bovina devem ser considerados em futuros estudos, principalmente, grupos defensores do bem-estar dos animais.

O manejo da dor requer colaboração de criadores, veterinários e técnicos veterinários. Entretanto, a percepção com relação à dor se distingue. Para os criadores, a redução da dor ao mínimo possível, em bovinos com problemas nos cascos, é significativamente importante se comparada aos especialistas, apesar de não serem a favor do uso de anestésicos no tratamento terapêutico (Becker et al., 2013). Também se identificou que os criadores, além de terem menor capacidade para reconhecer a dor, consideram os bovinos com aquele tipo de lesão menos sensíveis a dor que os veterinários (Becker et al., 2014).

O uso de mitigadores da dor em procedimentos dolorosos ainda é irrisório, até mesmo na castração e na descorna, considerados como os procedimentos mais dolorosos por veterinários neozelandeses (Laven et al., 2009). No final do século passado, estudo conduzido no Reino Unido relatou que 83% dos criadores raramente utilizava anestesia local na castração (Kent et al., 1996). Passados dez anos, pesquisa realizada no Reino Unido e na Irlanda do Norte, mostrou que o uso de analgésico é ínfimo pelos veterinários durante a castração cirúrgica (4,6%) e a descorna (1,7%) (Huxley & Whay, 2006). Com veterinários norte-americanos, os resultados não foram muito diferentes: na rotina, somente 22% disseram utilizar anestesia local prévia à castração e 21% disseram aplicar analgésico sistêmico no momento do procedimento (Coetzee et al., 2010b). Por outro lado, na Finlândia, 93% da amostra - estudantes do curso de Medicina Veterinária e veterinários – disseram que usariam sedação, anestesia local ou analgesia na descorna de bovinos (Norring et al., 2014).

Consenso entre os profissionais sobre o manejo apropriado a dor inexiste, já que as atitudes variam consideravelmente entre os indivíduos (Huxley & Whay, 2006; Laven et al., 2009; Phillips et al., 2009; Millman, 2013), e são influenciadas por características sociais e demográficas. Apesar de sutil, ao longo do tempo observou-se uma evolução nas atitudes conforme a idade (ano de graduação) do profissional: os mais jovens são mais propensos ao uso de analgésico e mais conscientes sobre os possíveis efeitos negativos caso o animal sinta dor (Huxley & Whay, 2006; Laven et al., 2009; Thomsen et al.,

2010). Também se percebeu que as mulheres veterinárias concordam mais com o argumento “o uso de analgésico permite uma recuperação mais rápida do animal” (Thomsen et al., 2010), além de darem maior escore a diversos procedimentos dolorosos (Huxley & Whay, 2006; Laven et al., 2009), que os homens. Já o tempo despedido no trato com os bovinos, exceto na castração cirúrgica (Laven et al., 2009), a universidade de graduação (Huxley & Whay, 2006), a experiência como veterinário e a afeição por uma animal de estimação da família (Norring et al., 2014) não influenciaram na estimação do escore da dor.

2.4 Procedimentos de manejo dolorosos na pecuária de corte

Procedimentos dolorosos comuns e empregados na bovinocultura de corte incluem a castração, a descorna e o amochamento, usualmente conduzidos em animais jovens e sem o uso de medicamento. A remoção, destruição ou degeneração dos testículos busca reduzir o comportamento agressivo, facilitar o manejo, prevenir acasalamento indiscriminado e melhorar a qualidade e a conformação da carcaça. Já a amputação ou a prevenção do crescimento dos cornos visa reduzir a ocorrência de lesões e ferimentos nos animais e nas pessoas, prevenir lesões na carcaça, além de permitir que maior número de animais tenha acesso ao comedouro (Hayward, 2002; Anderson, 2009; Stafford & Mellor, 2010).

Independente da prática, todos causam um forte estímulo mecânico, químico ou térmico devido à lesão tecidual, aplicada de forma aguda na pele, músculo ou víscera, provocando uma variedade de características comportamentais e mudanças na função autônoma (Short, 1998). Contudo, a descorna parece suscitar uma resposta aguda de maior intensidade e transitória, enquanto a castração parece causar uma resposta aguda mais prolongada (Morisse et al., 1995; Schwartzkopf-Genswein et al., 2005).

2.4.1 Castração

A castração é uma prática de manejo comum em sistemas de produção de bovinos de corte, sendo a idade e o método utilizado fatores que podem influenciar no nível de estresse (Robertson et al., 1994; Bretschneider, 2005; del Campo et al., 2014). Ademais, pode causar alterações fisiológicas, neuroendócrinas e comportamentais, inferindo dor e sofrimento (Fell et al., 1986; Stafford et al., 2002; Coetzee et al., 2008; Marti et al., 2010; Coetzee, 2013). Contudo, preocupação em mensurar a dor e o desconforto provocado pelo procedimento em animais de produção somente teve início nos anos 80 (Stafford & Mellor, 2005b).

Conforme diversos autores (Hayward, 2002; Stafford & Mellor, 2005b; Schwartzkopf-Genswein et al., 2012; AVMA, 2014), os métodos mais comuns são: i) castração física - cirúrgica, que é a retirada dos testículos depois de realizada incisão no escroto e não cirúrgica, a qual promove a constrição do suprimento de sangue aos testículos e escroto; ii) injeção química para induzir esclerose testicular; e iii) hormonal, que envolve a injeção de imunocontraceptivos para induzir a produção de anticorpos contra o hormônio liberador de gonadotrofina (GnRH). Este último método foi enfatizado por Guatteo et al. (2012) como uma alternativa possível e real para substituir o procedimento físico.

Diferentes métodos de castração foram avaliados por pesquisadores com o uso de indicadores comportamentais, de desempenho e pela concentração de cortisol. Fell et al. (1986) concluíram que a castração cirúrgica provoca uma reação mais severa que a não cirúrgica, comprovada pelos níveis de cortisol superiores entre 15 e 120 min. após o procedimento, sem alterar no ganho de peso (Fisher et al., 2001; Pang et al., 2008). Já Robertson et al. (1994), ao analisarem diferentes métodos não cirúrgicos, evidenciaram que bovinos castrados com anel de borracha demonstraram reação mais lenta e com Burdizzo uma dor mais intensa. Por fim, estudo conduzido por del Campo et al. (2014) evidenciou que a castração com faca tradicional provocou dor nos animais independente da idade e que o uso de anéis de borracha deve ser priorizado somente para bezerros com até um mês de idade. O

reconhecimento da dor pelos médicos veterinários neozelandeses concorda com as pesquisas anteriores: para os profissionais, a castração cirúrgica é a mais dolorida, seguida pelo Burdizzo e anel de borracha (Laven et al., 2009).

Bretschneider (2005) avaliou a influência da idade e mostrou que a resposta em bovinos castrados com idade inferior a seis meses tende a ser menor que a idades superiores, ou seja, quanto mais jovem é o animal, menor é o estresse. Em consonância, Currah et al. (2009) concluíram, mediante avaliação subjetiva, que com o aumento da idade também aumenta as possibilidades do animal ser classificado como “sentindo dor”.

Variedades de um método de castração bastante comum, mas questionável, a aplicação de anéis de borracha, foi estudada por inúmeros pesquisadores. Becker et al. (2012) avaliaram diferentes maneiras de aplicação, em animais entre quatro e seis semanas de idade, e deixaram claro que o uso de três borrachas provoca mais dor, promove inchaço prolongada e maior sensibilidade à palpação, sendo a aplicação de uma borracha e a remoção do saco escrotal nove dias após o procedimento o mais indicado, além de prático e fácil. Nas 8 h seguintes à castração com borracha, em animais que receberam anestésico ou anestésico e AINE, não houve diferença significativa na concentração plasmática de cortisol em relação ao valor pré-tratamento e em relação aos animais não castrados (Stafford et al., 2002). Já com a administração de analgésico e anestésico, em bovinos com três a quatro meses de idade, houve redução dos níveis de cortisol, da temperatura escrotal, do tempo de cicatrização, podendo haver redução do apetite e da atividade, sem influenciar no desempenho (González et al., 2010; Marti et al., 2010).

O uso de classes medicamentosas foi avaliado por diversos pesquisadores. Bezerros com 21-28 dias, castrados com Burdizzo ou anel de borracha, demonstraram redução dos indicadores de dor aguda, como níveis de cortisol, comportamento ativo e proporção de posturas anormais quando receberam anestésico local (Thüer et al., 2007). A combinação de anestesia local com analgesia, em bovinos com um mês de idade, diminuiu a resposta biológica do estresse (Stafford et al. 2002; del Campo et al., 2014). O uso de anestésico em animais com quatro meses de idade reduziu, mas não eliminou

completamente, a resposta ao estresse mensurada com a termografia infravermelha, com a variabilidade do ritmo cardíaco e com a concentração de cortisol (Stafford et al., 2002; Stewart et al., 2010). Em animais com 4-6 meses, a aplicação de uma dose subanestésica de α2-adrenérgico (xilazina) e anestésico (quetamina) reduziu significativamente a reação comportamental durante o procedimento e atenuou o nível de cortisol por 60 min. (Coetzee et al., 2010a). Já o uso do analgésico aspirina, por via oral na concentração 50 mg/kg, após a castração, falhou em reduzir o efeito agudo nos níveis de cortisol (Coetzee et al., 2007). Em bovinos castrados com 8 a 10 meses de idade, o uso de AINE antes da castração não influenciou o ganho de peso e o temperamento, mas reduziu a incidência de doença respiratória bovina no confinamento (Coetzee et al., 2012).

A necessidade de discussão para determinar até que ponto a dor causada pela castração é necessária e justificada, uma vez que isso irá determinar quando analgésicos deverão ou não ser utilizados na rotina (Stafford & Mellor, 2005b; Manteca, 2009). Por isso, aprovação de medicamentos seguros, convenientes e viáveis sob o ponto de vista econômico é fundamental. Só assim, será possível assegurar a implementação de estratégias para mitigação da dor como prática recorrente na bovinocultura de corte no momento da castração (Coetzee, 2011).

2.4.2 Descorna e amochamento

A prevenção do crescimento (amochamento) e a remoção dos cornos (descorna) são práticas realizadas em fazendas de gado de corte (Stafford & Mellor, 2005a), mesmo com a frequente seleção para animais mochos (Anderson, 2009; Stafford & Mellor, 2010). Apesar da finalidade dos procedimentos ser a mesma, eliminar ou diminuir o risco de agressão a pessoas e outros animais, as técnicas utilizadas são distintas e são conduzidos em idades diferentes – em animais adultos, a descorna; em bezerros, o amochamento. Por isso, a distinção entre os procedimentos, na literatura, não fica clara.

Independente da técnica – mecânica (fio serra, cabo descornador, faca, colher/tubo de descorna, descornador “Barnes” ou “Gouger”), por cauterização (ferro quente/termocautério) ou com o uso de produtos químicos -, todos causam redução do bem-estar e possíveis alterações comportamentais, fisiológicas e neuroendócrinas (Duffield, 2008; Anderson, 2009; Stafford & Mellor, 2010; Schwartzkopf-Genswein et al., 2012; Stock et al., 2013). Entretanto, devido ao crescimento do animal e ao aumento da base da área dos cornos, o procedimento em animais mais velhos é mais traumático (Duffield, 2008; Sinclair, 2012).

Stilwell et al. (2007) avaliaram os efeitos de métodos de remoção ou prevenção do crescimento dos cornos em diferentes idades - aos 117 dias, com colher; aos 98 dias, com termocautério; e aos 25 dias, com pasta cáustica – sobre a resposta comportamental e fisiológica. O método que causou dor mais intensa e por mais tempo foi o que utilizou a colher, coincidentemente, o realizado em animais mais velhos, os quais mostraram níveis de cortisol elevados e com maior incidência de alterações comportamentais por até 6 h após o procedimento. Entre dois e seis meses de idade, a descorna com ferro quente teve menor perda de sangue, mas a resposta comportamental em longo prazo foi cinco vezes superior em relação a descorna com faca ou colher, além da cicatrização ser mais lenta (Sinclair, 2012). Em animais com idade entre cinco e seis meses, independentemente do método utilizado para remoção dos cornos (com colher, fio serra e descornadeira - guilhotina ou serrote), o aumento do nível do cortisol é similar nas primeiras 6 h, apesar do comportamento dessa elevação ser variável (Sylvester et al., 1998).

O uso da cauterização na prevenção do crescimento dos cornos, procedimento comum na bovinocultura de corte, deve ser realizado em animais com até 12 semanas de idade e que tenham os cornos evidentemente palpáveis (Stilwell et al., 2010. Stafford & Mellor, 2011). O objetivo é promover a destruição da pele que contém as células queratogênicas na base dos cornos (Stafford & Mellor, 2011; Anderson, 2009). Animais sedados com α2-adrenérgico não tiveram as respostas comportamentais e a dor eliminadas, mas facilitou a administração de outro fármaco (Stilwell et al., 2010; Caray et

al., 2015). Ademais, quando combinado com anestésico local, houve redução da incidência de vocalização e da movimentação da cabeça e das orelhas (Stilwell et al., 2010); e quando associado ao AINE, observou-se redução de movimentos de escape durante o procedimento (Caray et al., 2015).

Stewart et al. (2008) observaram que a cauterização dos cornos sem o uso de anestésico local provocou maior temperatura ocular, provavelmente pela vasoconstrição periférica, e maior variabilidade do ritmo cardíaco, pela maior estimulação do sistema nervoso autônomo, nos primeiros 5 min. após o procedimento. O uso da anestesia regional prévia a cauterização foi capaz de reduzir a dor e o estresse durante e em até 2 h após o amochamento (Graf & Senn, 1999), sendo que a lidocaína a 5%, apesar de não prover nenhuma redução adicional na dor, provocou menos alterações comportamentais que a lidocaína a 2% (Doherty et al., 2007). O uso oral de AINE aliviou a dor, comprovado pelo aumento do apetite, já que os animais permaneceram mais tempo deitados e ao redor do cocho por até cinco dias (Theurer et al., 2012). Quando esse medicamento foi associado ao anestésico local, não houve efeito significativo no comportamento dos bezerros amochados com gás butano (Milligan et al., 2004). Já com termocautério elétrico, essa terapia multimodal mostrou-se eficiente na redução da movimentação frequente da cabeça e das orelhas (Faulkner & Weary, 2001).

O uso de produtos cáusticos busca destruir as células queratogênicas localizadas ao redor dos cornos, sendo indicado para bezerros com até três semanas de vida (Anderson, 2009; Stafford & Mellor, 2010). A administração intravenosa de um opiode de ação analgésica mostrou-se efetiva por somente 30 min. após o amochamento com pasta cáustica (Braz et al., 2012). Já em bovinos com oito semanas de idade, a concentração de cortisol retornou ao nível basal mais rápido quando utilizada a anestesia local preemptiva (Morisse et al., 1995). Em animais com quatro meses de idade, o uso de pasta cáustica para prevenção do crescimento dos cornos provocou dor intensa nos primeiros minutos e mudanças de comportamento por até 3 h (Stilwell et al., 2009). Esses mesmos autores identificaram que, mesmo com o

uso de anestésico regional, tanto o cortisol como movimentos frequentes da cabeça reduziram, mas não foram eliminados, durante a primeira hora.

Apesar de ser um método rápido e eficiente, a dor provocada pela descorna por amputação é significativa, sendo necessário a aplicação de fármacos para minimizá-la (Anderson, 2009; Stafford & Mellor, 2011). O uso de anestésico local em bovinos entre cinco e seis meses de idade reduziu as alterações comportamentais a níveis similares aos animais controle nas duas horas seguintes ao procedimento (Sylvester et al., 2004). Em bovinos com três a quatro meses de idade, o uso de anestésico regional em associação com AINE foi providencial para minimizar a dor (McMeekan et al., 1998; Stafford et al., 2003).

Normalmente, descorna mecânica provoca uma hemorragia que poderá ser intensa. Por isso, devem-se cauterizar os vasos sanguíneos seccionados ou, em casos mais extremos, administrar um anti-hemorrágico (Rodrigues , 1991). Quando o procedimento foi seguido da cauterização, houve aumento significativo dos níveis de haptoglobina, além do número de vocalizações e da agitação, impactando negativamente no bem-estar durante e após o procedimento (Sinclair, 2012). Contudo, conforme Sutherland et al. (2002), quando o anestésico regional é associado à cauterização, além de eliminar a perda de sangue, prolonga o período de alívio da dor além do ponto de atuação do fármaco isolado, podendo chegar a até 9 h em bovinos com idade entre cinco e seis meses de idade (Sylvester et al., 1998).

Devido aos problemas associados ao manejo com bovinos aspados e aos custos relativos a descorna e ao amochamento, a seleção genética para animais não aspados é uma maneira de minimizar os problemas de BEA, ainda mais que as diferenças fenotípicas pouco influenciam nas características produtivas (Goonewardene et al., 1999). Entretanto, por essa solução não ser prática e requerer tempo, pesquisas que busquem estratégias efetivas e viáveis para mitigar a dor, além de determinar qual método é menos doloroso, são fundamentais (Anderson, 2009; Duffield, 2008; Guatteo et al., 2012).

3. HIPÓTESES DO TRABALHO

A castração, a descorna e o amochamento em bovinos de corte provocam dor e impactam no bem-estar, alterando indicadores fisiológicos, comportamentais e de desempenho.

O uso de estratégias, farmacológicas ou técnicas, tendem a minimizar o sofrimento de bovinos de corte castrados, descornados ou amochados.

4. OBJETIVOS

4.1 Objetivo geral

O objetivo principal da tese é revisar, de forma sistemática, e quantificar, com o uso da meta-análise, dados disponíveis na literatura sobre três procedimentos dolorosos – castração, descorna e amochamento –, seus efeitos no bem-estar de bovinos de corte, além de alternativas para mitigar o sofrimento.

4.2 Objetivos específicos

- ✓ Descrever os procedimentos de castração, descorna e amochamento realizados em bovinos de corte com até um ano de idade;
- ✓ Quantificar, com o uso da meta-análise, o impacto de tais práticas em três indicadores de bem-estar animal: concentração de cortisol (parâmetro fisiológico), vocalização (indicador comportamental) e ganho médio diário de peso (medida de desempenho);
- ✓ Identificar, por meio da meta-regressão, fatores determinantes do aumento ou da redução da dor.

5. METODOLOGIA GERAL

A revisão sistemática (RS) e a meta-análise (MA) são importantes ferramentas na área da ciência animal. Conforme Lean et al. (2009), os achados obtidos de uma meta-análise rigorosa podem prover novos entendimentos sobre a ciência animal.

A RS baseia-se em uma revisão fundamentada em uma questão claramente formulada, que faz uso de métodos sistemáticos e explícitos para identificar, selecionar e avaliar, de maneira crítica, pesquisas relevantes, além de coletar e analisar dados de estudos que estejam incluídos na revisão. Dependendo da quantidade, diversidade, qualidade metodológica e descrição dos estudos primários, a MA pode ser uma etapa subsequente a RS. A MA faz referência à análise estatística oriunda de resultados complexos, muitas vezes contraditórios, permitindo uma análise com maior poder estatístico (Egger et al., 2001; Sargeant et al., 2005; Borenstein et al., 2009; Lean et al., 2009). Dessa maneira, permite transcender o resultado de análises anteriores, sendo uma reflexão crítica sobre elas e, por isso, considerada “a análise das análises” (Luiz, 2002).

Conforme demonstrado na Figura 3, alguns procedimentos constituem as boas práticas e compõem a engenharia da meta-análise, devendo ser aplicadas de modo mais rigoroso possível (Lovatto et al., 2007).

5.1 Manejo da revisão sistemática

5.1.1 Pergunta de pesquisa e protocolo

Essa revisão sistemática foi conduzida para identificar três procedimentos de manejo – castração, descorna e amochamento – e seus efeitos no bem-estar de bovinos de corte pela mensuração dos níveis de cortisol ou da vocalização ou do ganho de peso. O desenvolvimento do protocolo de revisão é consistente com guias publicados previamente (Sargeant et al., 2005; Higgins & Green, 2011). Tal protocolo foi desenvolvido com a contribuição de *experts* em produção e bem-estar animal. Em maio de 2013, o protocolo foi considerado finalizado.

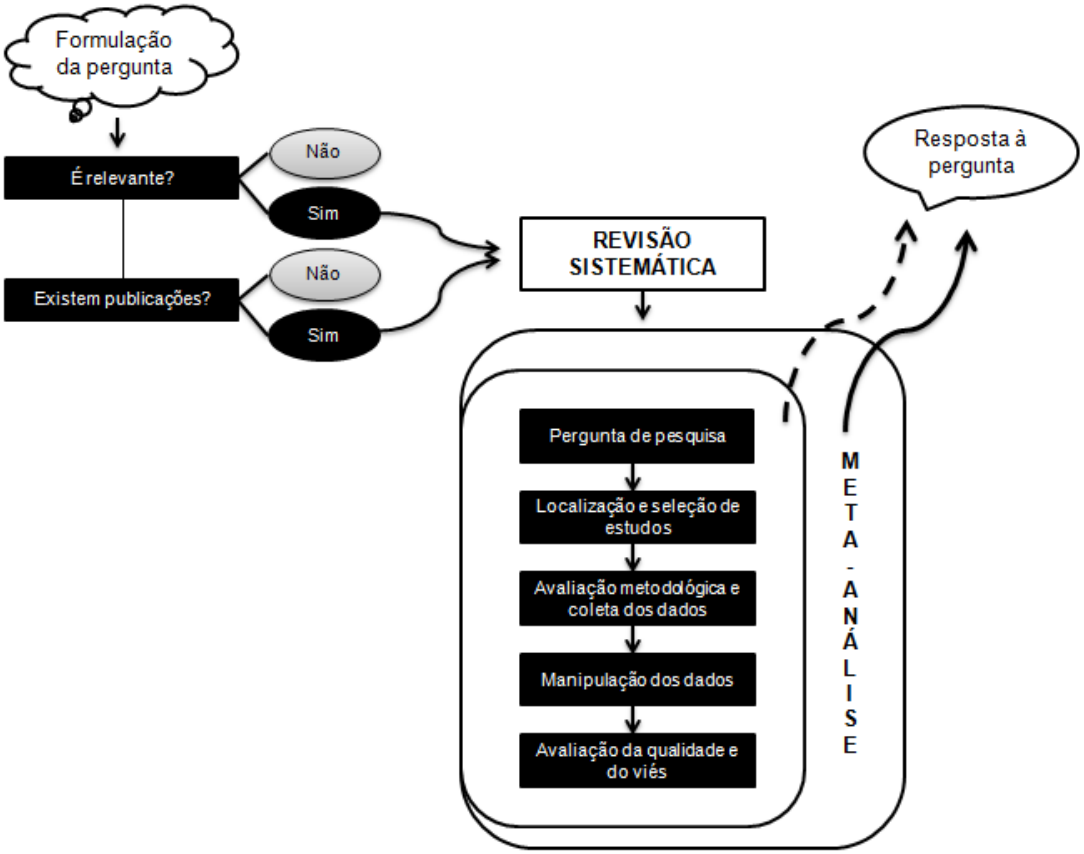

FIGURA 3. Fluxograma das diferentes fases da revisão sistemática e da meta-análise. Fonte: próprio autor.

Após sua finalização, uma mudança foi feita: a extração de dados de figuras passou a ser considerada, consequência da grande quantidade de dados que poderiam ser excluídos caso as informações não fossem utilizadas. Tal mudança poderá refletir nos resultados dessa pesquisa.

A pergunta de revisão foi definida no formato PICO: população (P), intervenção (I), comparador (C) e “desfecho”/resultado (O).

População

A população do estudo foi definida em dois componentes: espécie animal e idade. Estudos relevantes foram limitados aos bovinos de raças produtoras de carne com até 365 dias (um ano) de idade. Bovinos de corte, puros ou cruzados, descritos como neonatal, em aleitamento ou pré e pós-desmame foram considerados como parte relevante da população. Bovinos de raças leiteiras foram excluídos. Nenhuma outra restrição foi considerada com

relação aos animais (isto é, país, ano, sistema de produção ou ambiente de manejo).

Intervenção

Todos os tipos de intervenções relacionados com os procedimentos de castração, descorna e amochamento, com ou sem o uso de mitigadores de dor, foram considerados relevantes. Castração foi considerada qualquer ação – física, química ou hormonal – que busque remover, destruir ou reduzir a atividade dos testículos. Descorna como a amputação dos cornos em qualquer estágio após seu crescimento ter finalizado. Por fim, amochamento como a prevenção do crescimento antes de atingir um estágio avançado. Estudos em que os bovinos recebiam mais de um procedimento doloroso foram excluídos.

Comparador

Foram considerados grupos de comparação animais similares submetidos, ou não, ao mesmo procedimento. Não foram excluídos estudos com base no tipo de grupo controle utilizado. Por exemplo, animais castrados (com tratamento farmacológico ou não prévio a castração) ou “controle negativo” (animais são manejados como se o procedimento fosse conduzido, mas na realidade não o é) foram relevantes para o presente estudo.

Resultado

Resultados de interesse foram àqueles indicadores utilizados para acessar a dor nas três intervenções previamente descritas. O estudo deveria avaliar, pelo menos, um dos seguintes indicadores: cortisol (fisiológico), vocalização (comportamental) e ganho médio diário de peso / peso corporal (produtivo).

Um protocolo prévio foi desenvolvido e pré-testado. Cada instrumento de seleção para essa revisão foi adaptado de formulários previamente desenvolvidos (Mederos et al., 2012).

5.1.2 Estratégia de busca de literatura

Uma lista com os termos e algoritmos norteadores da pesquisa foi avaliada pela equipe de pesquisadores, a fim de assegurar que nenhum termo tenha sido esquecido. A lista final dos termos e algoritmos, sumarizada pelos componentes população, resultado e intervenção está na Tabela 3. Essa estratégia de pesquisa também auxiliou na busca por estudos relevantes que avaliassem medida de desempenho como resultado e, por isso, termos associados a desempenho animal (*average daily weight gain, performance, body weight*) não foram incluídos, a fim de evitar uma sobrecarga de citações não relevantes.

TABELA 3. Termos de busca final para população, resultado e intervenção na revisão sistemática.

Acrônimo	Descritores
População	bovine OR "beef cattle" OR cal* OR herd
Intervenção	disbud* OR dehorn* OR castration
Resultado	"animal wel*" OR "animal pain" OR "animal stress" OR cortisol OR behavio* OR vocali*

Cinco bases de dados eletrônicas foram utilizadas, em maio de 2013, e atualizadas em maio de 2015, por intermédio do *Instituto Nacional de Investigaciones Agropecuarias* (INIA Tacuarembó, Uruguai) e da Universidade Federal do Rio Grande do Sul (UFRGS, Brasil): CAB Abstracts (Thomson Reuters, 1910–2015), ISI Web of Science (Thomson Reuters, 1900–2015), PubMed (1940–2015), Agricola (EBSCO, 1970–2015) e Scopus (Elsevier, 1960–2015). Alguns resumos de conferências são indexados no CAB Abstract, sendo esperada sua captura pela estratégia de busca descrita acima. As principais conferências internacionais sobre etologia e produção animal - *International Society for Applied Ethology* (ISAE) and *ADSA-ASAS Joint Annual Meeting*, respectivamente -, entre os anos de 2001 e 2014, foram consideradas. Pesquisadores que atuam na área foram questionados quanto a dados não publicados. A verificação da busca incluiu uma pesquisa manual nas listas de referências de seis revisões de literatura que abordavam

procedimentos dolorosos e suas respostas em bovinos (Bretschneider, 2005; Stafford & Mellor, 2005a; Weary et al., 2006; Coetzee, 2011; Stafford & Mellor, 2011; Schwartzkopf-Genswein et al., 2012).

Todas as publicações foram importadas para o software Refworks (RefWorks–COS, USA) e as citações duplicadas foram removidas manualmente.

5.1.3 Critérios de seleção dos estudos e da triagem

Cinco revisores (estudante de doutorado, um pesquisador, um professor e dois estudantes de graduação) contribuíram nos diferentes níveis do processo de revisão, sendo previamente treinados para o processo de triagem a partir da avaliação de 30 resumos. As quatro questões de seleção optativas foram:

1. O resumo avaliado é um trabalho original?
2. Esse resumo avalia o bem-estar em bovinos de corte?
3. Esse resumo avalia castração, descorna ou amochamento em bovinos de corte?
4. Esse resumo mensura cortisol, vocalização ou ganho médio diário de peso (peso vivo) como indicadores de bem-estar em bovinos de corte?

Foram incluídos todos os estudos clínicos randomizados e não randomizados, estudo de coorte e casos-controle. Nessa etapa, nenhuma restrição de idioma e ano de publicação foi imposta.

Todas as referências identificadas foram avaliadas, de maneira independente, por dois revisores, mediante a leitura do título e do resumo (quando disponível). Quando ambos os revisores respondessem “não” em, ao menos, uma das questões acima, a referência era excluída. Diferenças de opiniões entre revisores foram solucionadas pelo consenso ou pela conferência dos dados originais e, se a discordância persistisse, um terceiro revisor era consultado.

Durante todas as etapas da RS, foi utilizado o formato de revisão

eletrônico SRSnexus (Möbius Analytics, Ottawa, Ontario, Canada)

5.1.4 Avaliação metodológica e processo de coleta dos dados

O formulário de extração dos dados, adaptado de outros estudos, foi aplicado a 102 artigos científicos. A pesquisadora principal foi a responsável pela extração dos dados dos artigos. Publicações com mais de um desenho experimental foram duplicadas e os dados extraídos como estudos separados, a fim de obter o máximo de detalhes possível.

Antes da avaliação do viés de publicação e da extração dos dados (ED), a relevância dos estudos selecionados pela avaliação do resumo foi confirmada com o uso do mesmo na íntegra: se o idioma de publicação era inglês, espanhol, português ou italiano; se possuía um grupo controle adequado; e se os resultados foram reportados com detalhes suficientes para conduzir a ED e a extração quantitativa de dados para meta-análise. Nessa etapa da revisão, os estudos originais foram limitados a publicações nestes quatro idiomas, já que esses eram os idiomas que os membros da equipe de pesquisa consideravam-se fluentes, e a tradução de artigos publicados em outros idiomas foi impedida devido ao elevado custo.

Informações extraídas de cada artigo foram estratificadas em gerais, população do estudo, intervenção, indicadores avaliados e resultado. Informações relativas ao artigo em si incluíram nome da revista científica, nome(s) do(s) autor(es), ano de publicação e idioma original.

Informações gerais incluíam: i) desenho experimental; ii) tipo de publicação (artigo científico original, anais de conferência, tese ou relatório/pesquisa governamental); iii) país/região/província/estado; iv) ano(s) da coleta dos dados; v) instituição de financiamento; vi) seleção das operações, rebanho/baia/piquete e bovinos (não reportado, randomizado, menção de randomização, sistemático, conveniência ou proposital, não aplicado); vii) tamanho da amostra individual e de fazendas; e viii) critérios de inclusão e exclusão.

Informações sobre população incluíam: i) local (não reportado,

propriedade rural comercial, fazenda de pesquisa, instalações de pesquisa); ii) gênero, raça e idade dos animais; e iii) número total de rebanhos, de bovinos e outros.

Informações de intervenção para cada grupo fazia referência ao tipo de procedimento (castração, descorna, amochamento, outro), quem realizou o procedimento (não reportado, funcionário rural, veterinário, outro), se algum medicamento foi utilizado para minimizar a dor, como a intervenção foi designada à unidade experimental (conveniência, randomizada, menção de randomização, sistemática), protocolo de intervenção, grupos de tratamento e se foi utilizado o cegamento durante a alocação dos tratamentos.

Para os indicadores avaliados foram observados: i) tipo de amostra; ii) se foi realizada mensuração do indicadores antes da intervenção (basal); iii) que tipo de método foi utilizado para avaliar o indicador; iv) cegamento para avaliar a resposta; v) agrupamento (não, sim, não aplicado); vi) identificação e controle dos fatores de confusão (não, sim/análise, sim/inclusão e exclusão, sim/comparação, não aplicado); e vii) se a análise estatística era reprodutível (não, sim, referência a outro artigo, não foi realizada análise estatística).

Com relação aos resultados, foram extraídas informações do tipo, unidade e tempo de mensuração para cada indicador. Especificamente, para dados brutos ou não ajustados buscou-se extrair média, desvio padrão (SD) ou outra medida de dispersão e valor da probabilidade. Sobre a análise estatística, foram incluídos: i) tipo de análise; ii) caso o modelo tenha sido ajustado, como?; iii) tipo de resultado (variável categórica ou contínua); iv) estimação do efeito de cada variável avaliada; v) avaliação da variabilidade do efeito estimado; e vi) valor da probabilidade e sua interpretação. Quando o resultado foi reportado como mensurado, mas os dados ou não foram reportados ou não foi possível extraí-los, os autores foram contatados.

5.1.5 Considerações sobre a coleta e a manipulação dos dados

Para cada um dos resultados de interesse, foram extraídos a média, o desvio padrão (SD) ou outra medida de dispersão, a unidade de medida, o

valor da probabilidade e o número de bovinos nos grupos controle e tratado. Quando necessário, os dados de cortisol e ganho médio diário de peso (GMD) foram convertidos para nmol/L e g/dia, respectivamente. Dados para vocalização foram obtidos em escala (0= ausência de vocalização; 1= bufando ou grunhindo; 2= vocalização momentânea; 3= vocalização durante e imediatamente após a manipulação do local do procedimento) ou na sua forma numérica.

Esse sumário de dados foi tabulado em planilhas eletrônicas do Microsoft Excel® e o banco de dados foi construído com base nos resultados dos estudos e dos resultados de interesse: cortisol (basal, 20 ou 30 ou 40 min. e 120 min.), GMD (durante o período de observação) e vocalização (durante a intervenção). Quando os resultados eram reportados na escala logarítmica, foram transformados para a sua escala original, conforme as fórmulas propostas por Mederos et al. (2012). Quando o desvio-padrão da média (SEMp) era reportado para os grupos controle e tratamento, o desvio-padrão agrupado (Sp) obtido foi derivado da fórmula (Ceballos et al., 2009):

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

onde n_p é o número de bezerros nos grupos avaliados.

Para àqueles estudos que reportavam somente o valor de probabilidade, a estimação de um desvio-padrão comum foi obtido com o uso de t-estatístico, assumindo que os dados tinham uma distribuição normal, com a aplicação da seguinte fórmula (Mederos et al., 2012):

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

onde x_2-x_1 representa a diferença entre as médias; $t(\alpha fdE)$ é o percentil da referida distribuição; e n é o tamanho da amostra de cada grupo.

Quando os dados eram apresentados somente na forma gráfica, um dos autores, preferencialmente o de correspondência, foi contatado via correio

eletrônico e questionado sobre a possibilidade de envio de um sumário dos dados estatísticos. Se não fosse obtida resposta ou se os dados não fossem fornecidos, a média e/ou medida de dispersão eram extraídos manualmente com o auxílio de uma régua.

Finalmente, como os dados de cortisol foram coletados em três diferentes momentos, o sumário desses dados foi recriado. Por fim, a medida de efeito foi computada segundo recomendações de Borenstein et al. (2009).

5.2 Avaliação da qualidade e do viés de publicação

Somente para os estudos que contribuíram com a meta-análise foi utilizado a Ferramenta de Risco de Viés da Colaboração Cochrane (*Cochrane Collaboration Risk of Bias Tool*) (Higgins & Green 2011) para avaliar o risco de viés de publicação nos estudos individuais. O viés de publicação foi acessado a partir dos seguintes critérios: i) geração de sequencia aleatória (randomização); ii) sigilo de alocação; iii) cegamento do pessoal; iv) cegamento dos avaliadores dos resultados; v) perda por seguimento; vi) descrição das perdas e exclusões; vii) interrupção precoce por benefício; e viii) relato seletivo de resultados.

Cada um desses critérios foi classificado como de risco baixo, alto ou incerto em produzir viés de publicação, conforme definições já estabelecidas (Higgins & Green, 2011), com pequenas modificações (detalhes abaixo). O viés, descrito a seguir, pode ser caracterizado como: i) e ii) viés de seleção, iii) viés de performance, iv) viés de detecção, v), vi) e vii) viés de atrito e viii) viés de relato.

5.2.1 Viés de seleção

Geração de sequência aleatória

Estudos que não descrevessem distribuição aleatória dos bezerros aos grupos foram classificados como tendo “risco incerto” de viés para este domínio. Para estudos que descrevessem uma distribuição aleatória, mas sem nenhuma descrição do processo, foi considerado como “baixo risco” de viés. Para estudos com descrição da distribuição aleatória (i.e. geração números randômicos por computador ou tabela de números randômicos), o viés foi

classificado como “baixo risco”.

Sigilo de alocação

Estudos que não descrevessem distribuição aleatória dos bovinos aos grupos foram classificados como tendo “risco incerto” de viés. Já para os estudos que descrevessem uma distribuição aleatória: i) se o método fosse descrito em detalhes que permitisse determinar se o sigilo de alocação poderia ter sido previsto antes ou durante a seleção, o risco de viés continuou sendo avaliado como “baixo risco”, “alto risco” ou “risco incerto” de viés com base na descrição feita por Higgins & Green (2011) e ii) se a descrição fosse insuficiente, o estudo foi descrito como tendo um “risco incerto” de viés.

5.2.2 Viés de performance

Para a avaliação do cegamento do pessoal foi averiguado se no estudo estavam descritas medidas que encobrissem os profissionais envolvidos de qual intervenção um participante recebeu e se o cegamento planejado foi efetivo. Se o método usado para cegar fosse descrito em detalhes suficientes para fazer uma determinação de sua eficácia, o risco de viés de performance continuou sendo avaliado como “baixo risco”, “alto risco” ou “risco incerto” conforme descrito por Higgins & Green (2011). Se a descrição fosse insuficiente, o estudo foi considerado como “risco incerto” de viés.

5.2.3 Viés de detecção

A relevância do cegamento foi avaliada com relação à avaliação do resultado. Primeiro, foi feito um julgamento sobre quais resultados eram considerados de “alto risco” ou “baixo risco” de viés de detecção caso não fosse cegado. Considerou-se que o resultado utilizado para mensurar o comportamento animal (vocalização) era de “alto risco” de viés se o cegamento não fosse informado e de “baixo risco” se fosse informado conforme descrito por Dzikamunhenga et al. (2014), já que esta é uma medida subjetiva e propensa a baixa fidedignidade (Weary et al., 2006). Indicador fisiológico (cortisol) e de desempenho (ganho médio diário de peso) foram considerados

de “baixo risco” de viés, independente da presença ou da ausência de cegamento.

5.2.4 Viés de atrito

O viés de atrito relata diferenças sistemáticas decorrentes da perda de dados de um resultado. Isso pode ser consequência da morte ou retirada de participantes de um grupo em relação ao outro. Se não há perda de dados dos desfechos, se a perda não está relacionada com o resultado investigado ou se inclui poucos dados perdidos para influenciar diferenças na medida de efeito o risco de viés foi considerado “baixo”. Por outro lado, quando a simples imputação dos dados é feita de maneira inapropriada ou quando as razões para a perda de dados estão relacionadas com o desfecho de interesse, podendo influenciar na medida de efeito observada ou provocar desequilíbrio na quantidade de participantes, considerou-se “alto risco” de viés. Para estudos com informação insuficiente, por ex., número randomizado não relatado ou com razões para perdas não descritas, o viés de atrito foi interpretado como de “risco incerto”.

5.2.5 Viés de relato

Para cada estudo, foi feita uma avaliação do risco de relato, que informa o viés que surge pela falta de inclusão de resultados não significativos. Uma classificação de “alto risco” foi dada quando ou era conhecido ou era suspeito de que os resultados não foram, ou foram parcialmente, reportados ou porque a comparação dos tratamentos não era significativo (P>0,05). Um viés de relato de “baixo risco” foi obtido quando foi suspeito, mas não realmente informado, de que o resultado não foi medido; medido, mas não analisado; ou foi medido e analisado, mas não foi ou foi parcialmente relatado por uma razão sem conexão com os resultados obtidos. Já o “risco incerto” foi reservado para casos em que se conhecia o resultado, i) mas o mesmo não foi mensurado, ii) mas o mesmo não foi analisado ou iii) sabia-se que estava sendo mensurado e analisado, mas a razão para a ausência de relato ou relato parcial não era porque os resultados não eram significativos.

5.3 Meta-análise

Estudos incluídos na análise quantitativa foram àqueles que reportassem resultados quantitativos suficientes para estimar a diferença padronizada de médias entre os grupos controle e tratado e seu intervalo de confiança a 95%. Para a concentração de cortisol, o valor obtido fez referência ao nível basal a 20/30/40 min. e a 120 min.; para GMD, durante o período de observação do estudo; para vocalização, durante o procedimento. Todas as análises foram realizadas com o pacote estatístico Stata v 14.0 (StataCorp., USA).

As transformações logarítmicas das médias e dos desvios-padrão dos grupos controle e tratado, antes da estimação da medida de efeito combinada da média e do desvio-padrão, foram realizadas de acordo com o método proposto por Higgins et al. (2008) para desvio-padrão separado por grupos.

Para o efeito aleatório da meta-análise foi considerada a pressuposição da existência de heterogeneidade entre os estudos. Tal heterogeneidade pode ser consequência de diferenças metodológicas e clínicas conhecidas entre os ensaios ou de características desconhecidas ou não registradas (Thompson, 2001). O método DerSimonian e Laird foi utilizado para estimar a heterogeneidade entre os estudos (DerSimonian & Laird, 1986).

Meta-análise por grupos de comparação

Foi conduzida uma meta-análise aparte, considerando vários subconjuntos de dados, formados por, pelo menos, dois estudos individuais que investigassem tratamentos similares, que usassem o mesmo desenho experimental e que analisassem o mesmo indicador. Simultaneamente, cada resultado foi avaliado separadamente como grupo, utilizando estratificação por técnica para conduzir o procedimento e estratégia utilizada para minimizar a dor.

Foi gerada a medida de efeito combinada e o intervalo de confiança a 95% (*forest plot*). O Q de Cochran (teste de heterogeneidade qui-quadrado) e

I^2 (porcentagem de variação total entre estudos que é devido a heterogeneidade e não ao acaso) foram calculados com base no método e no resultado. A magnitude do I^2 foi interpretada na ordem de 25, 50 e 75%, percentuais que podem ser considerados como baixa, moderada ou alta heterogeneidade (Higgins et al., 2003). Diferença foi considerada significativa quando P<0,05 e com tendência quando 0,05≤ P <0,1.

5.4 Viés de publicação

O viés de publicação foi avaliado visual e estatisticamente com o uso do *funnel plot* e dos testes de correlação de Begg's e regressão linear de Egger's para cada indicador (cortisol, GMD e vocalização), incluindo todos os grupos de tratamento. Foi considerada a presença de viés se ao menos um dos métodos estatísticos fosse significativo (P<0,10). Se alguma evidência de viés de publicação estivesse presente, seja pelas análises estatísticas ou pelo gráfico em funil, os métodos "trim e fill" (Duval & Tweedie, 2000), foram usados para estimar a quantidade e a magnitude dos estudos perdidos, além do efeito estimado resultado para o viés. Com a aplicação desse último método, é possível estimar o "centro" do funil, estudos omitidos podem ser repostos e estudos perdidos recolocados ao redor da região central (Sterne et al., 2001).

5.5 Meta-regressão

O modelo de regressão para efeitos aleatórios permite explorar as fontes da heterogeneidade, sendo que uma regressão com base nas características dos estudos pode ser uma fonte de variação e influenciar na resposta ao tratamento (Borenstein et al., 2009; Lean et al., 2009).

Por isso, as variáveis exploradas na meta-regressão univariável foram: (1) randomização (não ou sim), (2) agrupamento (não, sim ou não aplicado), (3) identificação e controle de fatores de confusão (não, sim ou não aplicado), (4) ano de publicação, (5) tipo de publicação (artigo científico original, anais de conferência, tese ou relatório/pesquisa governamental), (6) continente (América do Norte, América do Sul, Europa, Ásia ou Oceania), (7) subespécie dos bovinos (*Bos taurus*, *Bos indicus*, híbrido/misto ou não

informado), (8) quem realizou a intervenção (não reportado, funcionário rural ou veterinário), (9) gênero (fêmea, macho ou macho e fêmea), (10) uso de medicamento para mitigar a dor (não ou sim), (11) tipo de medicamento utilizado (não aplicado, analgésico-sedativo, anestésico, anti-inflamatório ou terapia multimodal), (12) método utilizado (na castração: cirúrgico, não cirúrgico ou cirúrgico *vs.* não cirúrgico, na descorna e amochamento: amputação ou cauterização), (13) idade dos animais, (14) período de seguimento e (15) tamanho de amostra.

5.6 Meta-análise acumulativa e Análise de sensibilidade

Na meta-análise acumulativa, a medida de efeito global é atualizada a cada momento em que resultados de um novo estudo são publicados. Essa análise é conduzida, com maior frequência, com o sorteio cronológico dos estudos, permitindo identificar o momento em que o efeito do tratamento foi significativo em relação ao controle (Egger et al., 2001; Borenstein et al., 2009).

A análise de sensibilidade foi realizada para determinar se determinados estudos possuem impacto na medida de efeito. Tal análise foi realizada a partir da retirada manual de um estudo por vez e avaliação se a diferença entre as médias alterou-se em ±30%, antes da inserção desse estudo com posterior remoção do próximo, e assim sucessivamente.

CAPÍTULO II[1]

"Happiness is when what you think, what you say, and what you do are in harmony"
(Mahatma Ghandi)

[1] Manuscrito elaborado conforme as normas da Journal of Animal Science (Apêndice 3).

A meta-analysis of welfare indicators associated with castration in beef cattle[1]

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ABSTRACT

Castration of cattle is a common livestock management practice, although considered a painful procedure. Results from experimental studies suggest that pain can be reduced in various ways. However, the evidences are not fully conclusive. We aimed to assess the effects of castration in male beef cattle on welfare indicators (cortisol concentration, ADG, and vocalization). We systematically searched on five electronic databases up to May 2015, and conference proceedings, as well as we contacted experts. The main inclusion criteria were complete and non-randomized studies using beef cattle until one year of age, undergoing castration that reported cortisol concentration or ADG or vocalization as the outcome. Data were extracted using pre-defined protocols. Random effect meta-analyses (MA) were conducted for each indicator separately with the mean of control and treated groups. A total of 18 prospective publications reporting 23 studies and 156 trials were included in the MA involving 1,617 cattle. Significant heterogeneity between studies was observed for cortisol and ADG. Regardless the control group, the comparison analyses showed no significant ($P \geq 0.05$) changes on cortisol when castration was performed without pain management. Multi-modal therapy did not decrease ($P \geq 0.05$) cortisol concentration 30 min after non-surgical castration. From the MA comparing surgical castration with and without anaesthesia there was a tendency ($P = 0.077$) to decrease cortisol levels 120 min after intervention. Meta-analysis from studies measuring ADG as an outcome showed an increase of 0.883 g/d and 0.231 g/d only for non-surgical ($P = 0.002$) and surgical castration ($P = 0.010$), respectively, with no pain mitigation when compared to a non-castrated group. Publication bias was observed for results when cortisol was studied as an outcome. Only publication type contributed to explain the total variation between studies (18.52%) when the outcome

measured was ADG. Results from this study are not conclusive to draw recommendations on preferred castration practices to minimize pain in beef cattle.

Key words: animal welfare, cattle, cortisol, pain, performance, vocalization

INTRODUCTION

Castration is a common livestock procedure used to reduce management problems in animal husbandry. Physical procedure is the most commonly used, even though it increases cortisol concentration and changes in behavioural measures (Fell et al., 1986; González et al., 2010). For this reason, a hormonal method has been proposed as an animal welfare friendly alternative (Martí, 2012).

The attention in the pain caused by routine husbandry practices has increased. This has motivated the performance of research into the evaluation of pain and how it can be relieved (Stafford and Mellor, 2005). The current state of knowledge concerning the castration methods and pain management, together with highlights areas where there is insufficient evidence and need for future research, have been discussed in a subjectively manner by narrative reviews (Stafford and Mellor, 2005; Coetzee, 2011). The systematic review (SR) provides a rigorous and replicable method to increase the credibility to findings in the studied field. Depending on the quantity, diversity, methodological soundness and reporting of the primary studies, meta-analysis (MA) could be a subset of SR. Meta-analysis refers to the statistical synthesis of results from a complex, sometimes apparently conflicting, body of literature, but it can never prevent biases (Egger et al., 2001; Borenstein et al., 2009; Lean et al., 2009)

Lean et al. (2009) reported that a rigorously conducted MA is a useful tool to improve animal well-being and productivity. Then, we conducted a SR-MA to test the hypothesis that, although the castration is a painful procedure, strategies recognized it

could be used to prevent or minimize the adverse impacts on the welfare of cattle. The purpose of the study reported here was to identify, evaluate, critically appraise and synthesize, with a systematic review-meta-analysis approach, available literature reporting how castration procedures affect the beef cattle welfare.

MATERIAL AND METHODS

Research question and protocols

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

The search strategy was defined based on key concepts in terms of PICO format: population (P), intervention (I), comparator (C), and outcome (O). The study population was beef cattle until 12 months of age. Studies were included if the interventions were castration, dehorning, and disbudding. The present study only discusses castration procedure, while in a second stage the analysis concerns disbudding and dehorning interventions. Comparison groups of interest were similar animals undergoing the same procedure with or without intervention. We did not exclude publications based on the type of comparison used. Outcomes of interest were vocalization, cortisol, and ADG (see Appendix Table A1).

The research team collapsed the castration methods into three groups: 1) surgical castration including Newberry knife, knife, and scalpel (plus emasculator or Henderson Castrating tool[*]); 2) non-surgical castration including elastrator rings, band, Callicrate

[*] The castration tool is clamped in each spermatic cord individually and rotated by a cordless drill approximately 20 rotations until the cord severed.

bander[†], Burdizzo emasculator (plus elastrator rings), and immunocastration; and 3) surgical vs. non-surgical castration. Also, relevant pain mitigation were identified as analgesic-sedative (xylazine), anaesthesia (lidocaine, and combination of xylazine and ketamine), anti-inflammatory (dexamethasone, dypirone, ketoprofen, and meloxicam), and multi-modal therapy (combination of xylazine and flunixin or procaine, and lidocaine and dypirone).

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

Search methods for identification of studies

A list of final search terms and algorithms was summarized by population, outcome and intervention components of the question as follow: (bovine OR "beef cattle" OR cal* OR herd) AND (disbud* OR dehorn* OR castration) AND (“animal wel*" OR "animal pain" OR "animal stress" OR cortisol OR behavio* OR vocali*). These search strategy also retrieved relevant studies evaluating animal performance as the outcome, therefore “average daily weight gain” was not included to avoid the overload of non-relevant citations.

A systematic literature search was conducted on five electronic databases – CAB Abstracts (Thomson Reuters, 1910–2015), ISI Web of Science (Thomson Reuters, 1900–2015), PubMed (1940–2015), Agricola (EBSCO, 1970–2015) and Scopus (Elsevier, 1960–2015) – and were searched on May 2013 and updated on May 2015. In addition, the following proceedings were searched for references: ADSA-ASAS Joint

[†] The band is applied around the scrotum proximal to the testes. The elastic band is tightened until adequate tension, a metal grommet was then crimped around the band to hold tension.

Annual Meeting (from 2001 to 2014) and International Society for Applied Ethology, ISAE (from 2001 to 2014). We also electronically contacted investigators to request unpublished data. Reference search verification was performed by searching the reference lists from four recent literature reviews (Bretschneider, 2005; Weary et al., 2006; Coetzee, 2011; Schwartzkopf-Genswein et al., 2012).

All citations were imported into the reference manager Refworks (RefWorks–COS, USA) and duplicate citations were removed manually.

Study selection criteria and relevance screening

A total of five reviewers contributed to the different levels and were trained for the relevance screening step using 30 abstracts during pre-test.

The citation was considered as relevant when investigated primary research; animal welfare in beef cattle; castration, dehorning, or disbudding as an interventions; and measure cortisol, vocalization, or ADG as a welfare indicators.

The included study designs were randomized and non-randomized clinical trials, cohort studies, and case-controls. At this stage, no limits were applied for language or year.

All identified citations were independently assessed for relevance by two independent reviewers using the titles and abstracts (when available) and conflicts were resolved with consultation and when agreement was not attain, an expert opinion was requested.

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

Methodological assessment and data collection process

Data extraction forms were adapted and piloted on 102 publications. The first author was responsible for extracted data from the eligible studies. Publications reporting more than one study design were duplicated and extracted as separate studies.

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

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

Considerations for data collection and data manipulations

For each outcome, we attempted to extract the mean, standard deviation (SD) or any available measure of dispersion, measurement unit, *P*-value, and the number of animals in control and treatment groups. Cortisol and ADG data were converted to nmol/L and g/d, respectively. In the database, data from vocalization was on a scale of 0 to 3 (0 = no vocalization; 1 = snorting or grunting; 2 = momentary vocalization; and 3 = continuous vocalization during and immediately after testicular manipulation). These summary measures were entered into an electronic spreadsheet and a dataset was built containing the results for controlled studies, measuring outcomes of interest: cortisol (baseline, 20

or 30 or 40 min, and 120 min), ADG (during observation period) or vocalization scores (during procedure). When the results were reported in the log-transformed scales, these were transformed back to the original scale using the formula described in Mederos et al. (2012). Whenever an overall standard error of the mean (SEMp) was reported for the control and treatment groups, a pooled standard deviation (Sp) was derived from the formula (Ceballos et al., 2009):

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

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

For those studies that reported only *P*-values, an estimate of a common standard deviation was computed using the t-statistic, assuming the data were normally distributed, using the formula (Mederos et al., 2012):

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

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

When results were only graphically presented, the corresponding author was contacted by electronic mail and asked to provide the summary statistics. If no response was obtained or data were not provided, the mean or measure of dispersion or both were extracted by manual measurement using a ruler.

Finally, as the cortisol data were collected in those three different points, the summary data were recreated and then the effect size was computed according to recommended approaches (Borenstein et al., 2009).

Quality assessment

We used standardized methods to analyse the risk of bias of the individual studies included in the MA (Higgins and Green, 2011), with one minor modification. To evaluate the domain "blinding of outcome assessment", we considered that the behavioural outcome was at high risk of bias if blinding was not reported and at low risk of bias if blinding was reported, as described by Dzikamunhenga et al. (2014), once it is a subjective measure and more prone to poor reliability (Weary et al., 2006). Physiological and performance outcome were considered to be at low risk of bias regardless of the presence or absence of blinding.

Meta-analysis

Studies were included in the quantitative analysis when they reported sufficient data to estimate a standardized mean difference (MD) between control and treatment groups and its 95% confidence interval. For cortisol, the value obtained from baseline to 20/30/40 min and to 120 min was analysed, while for ADG it was during observation period. All the analyses were performed in the statistical package Stata V 14.0 (StataCorp., Texas, USA).

The random effect MA and meta-regression were carried out given the *a priori* assumption that between-study heterogeneity was present. The DerSimonian and Laird method was used to estimate the between study variation (DerSimonian and Laird, 1986).

Comparison groups meta-analysis. A separate MA was conducted using various subsets of data, consisting of at least two individual studies that investigated similar treatments and the same outcome. Concurrently, each outcome was evaluated separately as a group using stratification by castration method and pain management. A pooled

MD and 95% CI were generated (forest plots). The Cochran's Q (a chi-squared test of heterogeneity) and I^2 (percentage of total variation between studies that is due to heterogeneity rather than chance) were calculated based on the castration method and outcome. The magnitude of I^2 was interpreted in the order of 25%, 50%, and 75% which might be considered as low, moderate, or high heterogeneity (Higgins et al., 2003). Differences were considered significant at $P < 0.05$ and trends were defined at $0.05 \leq P < 0.1$.

Publication bias

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

Meta-regression

Random-effects regression univariable models were performed to evaluate sources of between-study heterogeneity that may influence the response of subjects to treatment (Borenstein et al., 2009). Meta-regressions were carried out for trials reporting cortisol concentration and ADG as outcomes by using method-of-moments estimator.

The variables explored in the meta-regressions were (1) randomization (no or yes), (2) cluster (no, yes or not applicable), (3) confounders identified and controlled (no, yes or not applicable), (4) publication year, (5) publication type (peer-reviewed, conference proceedings, thesis, government or research stations reports), (6) continent (North

America, South America, Europe, Asia or Oceania), (7) cattle group (*Bos taurus*, *Bos indicus*, hybrid/mixed or not reported) (8) who performed the intervention (not reported, farm staff or veterinarian), (9) application of medicine for pain relief (no or yes), (10) type of medicine (not applicable, analgesic-sedative, anaesthesia, anti-inflammatory or multi-modal therapy), (11) method of castration (surgical, non-surgical or surgical vs. non-surgical), (12) cattle age, (13) intervention follow-up period, and (14) sample size.

Cumulative meta-analysis and Influential studies

A cumulative meta-analysis was conducted to evaluate the pooled estimate of the treatment effect each time the result of a potential new study is published. Those analyses are most often used to display the pattern of the evidence over time (Borenstein et al., 2009).

Sensitivity analyses were performed to determine whether certain studies had substantial impact on the MD by manually replacing and removing one study at a time and evaluating whether the effect had change by ±30%.

RESULTS

Study selection and characteristic

Search identified 1,267 publications (Figure 1). Out of 102 full-text publications assessed for eligibility, 69 were excluded after methodological soundness and data extraction. Out of the remaining, 10 publications had not enough data to perform the quantitative analysis (see Appendix Table A2), and 18 were included in the SR-MA about castration (Table 1).

From 20 contacted authors who presented their results graphically or without sufficient data, numerical data were obtained from two (one from the USA and one from Uruguay).

The treatment groups evaluated in the study were surgical castration (n = 16 studies), non-surgical castration (n = 15), and non-surgical vs. surgical castration (n = 12). Relevant pain mitigation included one study that analysed analgesic-sedative, seven evaluating anaesthesia, six evaluating anti-inflammatory, and four evaluating multi-modal therapy.

The total number of cattle for the included studies that evaluated castration methods and cortisol concentration, ADG, and vocalization were 402, 1,451, and 32, respectively.

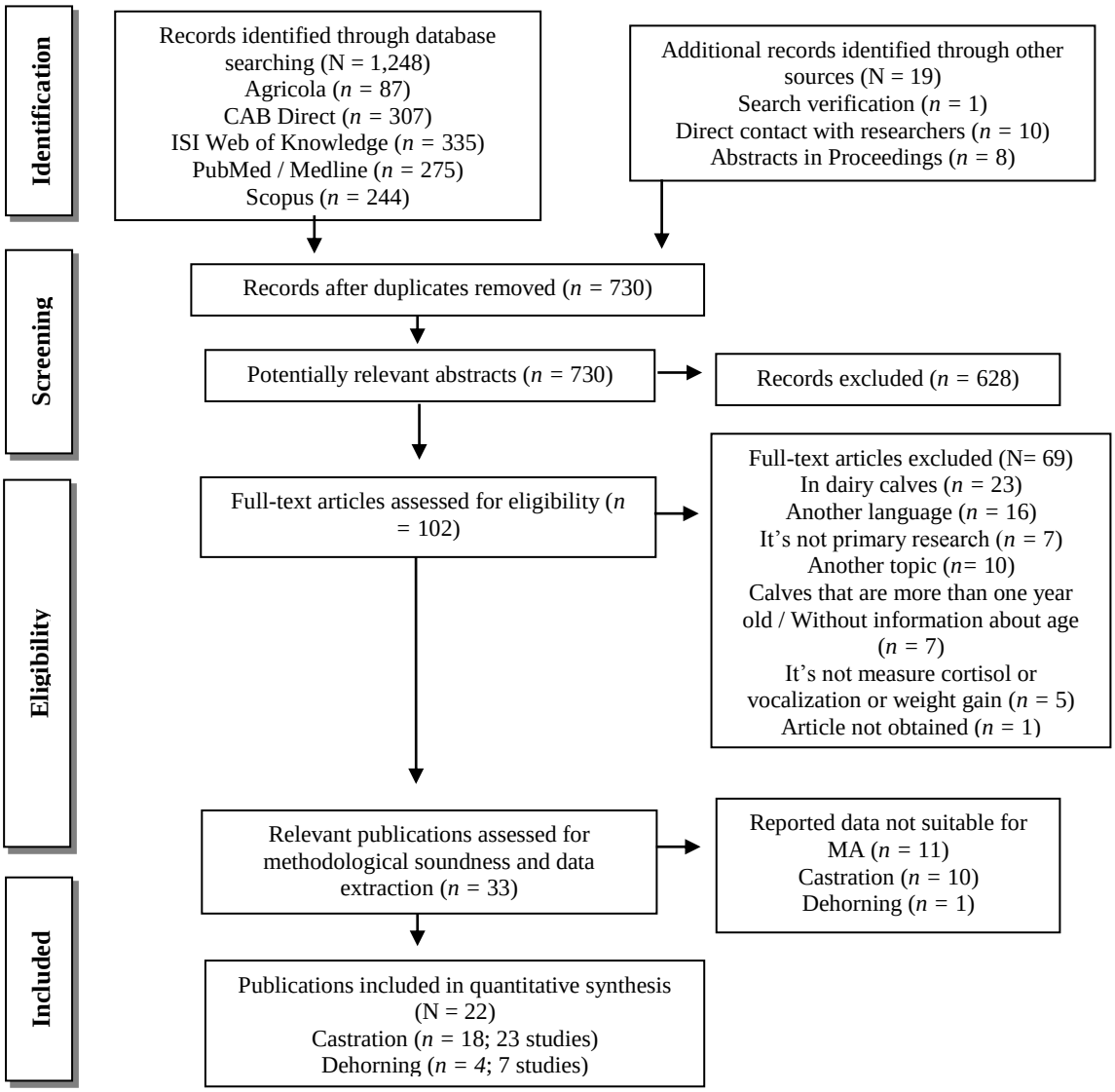

Figure 1. Flow diagram indicating the data recording in systematic review with the number of abstracts and publications included and excluded in each level. MA: meta-analysis. Adapted from PRISMA guidelines (Moher et al., 2009).

Table 1. A descriptive summary of each relevant study that was included in the final systematic review and was used in the meta-analysis and meta-regression (23)

Reference	Country	Study population (age in days / sample size)	Comparison groups	Analgesic regimen	Outcome parameter
Fell et al., 1986	Australia	49 / 27	Non-surgical vs. Surgical	NA[1]	Cortisol (30 and 120 min)
Faulkner et al., 1992	USA	240 / 268	Surgical	NA	ADG (27 d)
Baker et al., 2000	USA	108 / 60	Surgical, non-surgical and non- surgical vs. surgical	NA	ADG (217 d)
Fischer et al., 2001	New Zealand	270 / 40	Surgical, non-surgical and non- surgical vs. surgical	Anaesthesia	ADG (56 d)
Thüer et al., 2007	Switzerland	24 / 50	Non-surgical	Anaesthesia	Cortisol (30 and 120 min)
Coetzee et al., 2008	USA	150 / 10	Surgical	NA	Cortisol (30 and 120 min)
			Surgical	NA	Vocalization (during procedure)
Pang et al., 2008	Ireland	360 / 243	Non-surgical	Anaesthesia	ADG (56 d)
Coetzee et al., 2010	USA	150 / 22	Surgical	Analgesic-sedative and Anaesthesia	Cortisol (30 and 120 min) Vocalization (during procedure)
González et al., 2010	Canada	34 / 89	Non-surgical	Multi-modal therapy	Cortisol (30 and 120 min) ADG (42 d)
Petherick et al., 2011	Australia	255 / 32	Surgical, non-surgical and non- surgical vs. surgical	Anti-inflammatory	Cortisol (30 and 120 min)

					ADG (84 d)
Coetzee et al., 2012	USA	270 / 250	Surgical	Anti-inflammatory	ADG (28 d)
Warnock et al., 2012	USA	223 / 75	Surgical, non-surgical and non- surgical vs. surgical	NA	ADG (84 d)
Petherick et al., 2012	Australia	180 / 28	Surgical, non-surgical and non- surgical vs. surgical	NA	Cortisol (30 and 120 min)
Petherick et al., 2012	Australia	90 / 29	Surgical, non-surgical and non- surgical vs. surgical	NA	Cortisol (30 and 120 min)
Martí, 2012	Canada	257 / 60	Non-surgical	NA	Cortisol (30 and 120 min)
					ADG (56 d)
Reppening et al., 2013	USA	336 / 20	Non-surgical vs. Surgical	NA	ADG (28 d)
Pieler et al., 2013	Austria	56 / 40	Surgical, non-surgical and non- surgical vs. surgical	Multi-modal therapy	Cortisol (30 and 120 min)
					ADG (8 d)
Whitlock et al., 2013	USA	300 / 48	Non-surgical	Anti-inflammatory	ADG (28 d)
del Campo et al., 2014	Uruguay	7 / 36	Surgical, non-surgical and non- surgical vs. surgical	Anaesthesia	Cortisol (120 min)
					ADG (42 days)
del Campo et al., 2014	Uruguay	30 / 54	Surgical, non-surgical and non- surgical vs. surgical	Anti-inflammatory and multi-modal therapy	ADG (63 d)
del Campo et al., 2014	Uruguay	360 / 36	Surgical	Anaesthesia	Cortisol (120 min)
					ADG (42 d)
del Campo et al., 2014	Uruguay	360 / 40	Surgical	Anti-inflammatory and multi-modal therapy	ADG (28 d)
del Campo et al., 2014	Uruguay	360 / 60	Surgical, non-surgical and non- surgical vs. surgical	Anaesthesia and anti-inflammatory	ADG (56 d)

[1]NA: not applicable.

In total, 18 publications were included in this SR-MA which comprised 23 studies and 156 unique treatment comparisons. The results of the main characteristics of the included studies are presented in Table 2.

Table 2. Descriptive characteristic of 18 publications reporting 23 studies which were included in the systematic review-meta-analysis

Variable	**Description**	**Categories**	**Number of publications (studies)**
Study design	Type of study design used	Control studies	18 (23)
Publication type	Type of literature the work was published	Peer-reviewed	13 (13)
		Conference proceedings	1 (1)
		Thesis	1 (1)
		Government or research station report	3 (8)
Treatment	Type of procedure evaluated	Surgical castration	11 (16)
		Non-surgical castration	12 (15)
		Non-surgical vs. Surgical	9 (12)
Data published	Year of study publication	1990-2000	3 (3)
		2001-2015	15 (20)
Medicament	It was used any class of medicament?	No	14 (19)
		Yes	10 (14)
Medicament	If was used any medicament to mitigate pain, which class?	Analgesic-sedative	1 (1)
		Anaesthesia	5 (7)
		Anti-inflammatory	4 (6)
		Multi-modal therapy	3 (4)
Cattle group	Cattle group in which interventions were evaluated	*Bos taurus*	8 (9)
		Bos indicus	0 (0)
		Hybrid / Mixed	10 (12)
		Not reported	2 (2)
Who performed	Who performed procedure	Farm staff	5 (6)
		Veterinarian	10 (14)
		Not reported	3 (3)
Outcome	Parameter used to assess pain	Average daily gain	13 (17)

assessed	in calves		
		Cortisol	10 (12)
Sample size	Size of total study population per study	n≤50	11 (14)
		n= 51-100	4 (6)
		n≥101	3 (3)
Continent		North America	10 (10)
		South America	1 (5)
		Europe	3 (3)
		Asia	0 (0)
		Oceania	4 (5)

Risk of bias

Several studies failed to give enough detail to assess the potential risk of bias as presented in Table 3 and Appendix Table A3.

None of the studies provided sufficient details about the blind of personnel, and then the risk of performance bias was unclear. The risk of detection bias was considered relevant only for vocalization, and none of the studies used to blind outcome assessor from knowledge of which intervention a participant received, making the risk of detection bias high. The approach to describe the completeness of outcome data for each main outcome showed high risk of bias in two studies that evaluated cortisol (Petherick et al., 2012). Both gave a reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention group.

Table 3. Methodological quality assessment for risk of bias (classified as low, unclear, and high) of the 23 studies included in the systematic review of welfare animal in 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 Vocalization	Unclear Unclear	Low High	Low Low
Pang et al., 2008	Low	Unclear	Low	ADG	Unclear	Low	Low
Coetzee et al., 2010	Low	Low	Unclear	Cortisol Vocalization	Unclear Unclear	Low High	Low Low
González et al., 2010	Low	Unclear	High	Cortisol ADG	Unclear Unclear	Low Low	Low Low
Petherick et al., 2011	High	High	Low	Cortisol ADG	Unclear Unclear	Low Low	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 ADG	Unclear Unclear	Low Low	Low Low
Reppening et al., 2013	High	High	Low	ADG	Unclear	Low	Low
Pieler et al., 2013	Low	Unclear	Low	Cortisol ADG	Unclear Unclear	Low Low	Low Low
Whitlock et al., 2013	Low	Unclear	High	ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	Cortisol ADG	Unclear Unclear	Low Low	Low Low
del Campo et al., 2014	Unclear	Unclear	High	ADG	Unclear	Low	Low
del Campo et al., 2014	Unclear	Unclear	High	Cortisol ADG	Unclear Unclear	Low Low	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

Effect of castration on vocalization: descriptive analysis

The vocalization score was the less investigated outcome, and data were presented in a manner that was not usable in MA. Therefore, the available vocalization data was summarized and presented in Table 4. Changes in behaviour score did not show association with castration, regardless the use or not of some strategy to relief the pain

Table 4. Summary of the scientific literature examining the effect of castration in vocalization scores

Reference	Comparison groups	n	Mean	SD	Significance (p-value)
Coetzee et al., 2008	Uncastrated vs. Surgical	10	0.3 vs 1.8	1.2	0.12
	Uncastrated vs. Surgical + Analgesic-sedative	10	0.3 vs 1.2	0.7	0.12
	Uncastrated vs. Surgical + Anaesthesia	10	0.3 vs 0.6	0.2	0.12
	Surgical vs. Surgical + Analgesic-sedative	12	1.8 vs. 1.2	0.6	0.12
	Surgical vs. Surgical + Anaesthesia	12	1.8 vs.0.6	1.1	0.12
	Surgical + Analgesic-sedative vs. Surgical + Anaesthesia	12	1.2 vs. 0.6	0.6	0.12
Coetzee et al., 2010	Uncastrated vs. Surgical	10	0.2 vs. 2.2	1.4	0.052

Meta-analysis

Only cortisol concentration and ADG data were submitted to MA. One hundred and fifty six trials from 23 studies were included. There were no exclusions due to lack of randomization procedures or lack of adjusting for clustering and confounders. The numbers of publications, studies, trials, and type of outcome measurements available for the statistical analyses contained in the dataset are presented in Table 5.

Table 5. Number of publications in meta-analysis and/or meta-regression, stratified by procedure, outcome, and the use of medicament

Medicament	Publication (studies)	Studies (trials) ADG	Cortisol
	Non-surgical castration		
No	7 (10)	8 (15)	4 (11)
Yes	7 (7)	5 (10)	4 (9)
Analgesic-sedative	0 (0)	0 (0)	0 (0)
Anaesthesia	3 (3)	2 (4)	1 (4)
Anti-inflammatory	2 (2)	1 (2)	1 (2)
Multi-modal therapy	2 (2)	2 (4)	2 (3)
Total	*12 (15)*	*11 (25)*	*8 (20)*
	Surgical castration		
No	9 (13)	9 (12)	6 (10)
Yes	6 (10)	8 (30)	5 (20)
Analgesic-sedative	1 (1)	0 (0)	1 (4)
Anaesthesia	3 (5)	4 (8)	3 (10)
Anti-inflammatory	3 (5)	4 (12)	1 (2)
Multi-modal therapy	2 (3)	3 (10)	1 (4)
Total	*11 (16)*	*11 (42)*	*8 (30)*
	Non-surgical vs. Surgical		
No	7 (10)	7 (10)	5 (9)
Yes	4 (6)	5 (12)	3 (8)
Analgesic-sedative	0 (0)	0 (0)	0 (0)
Anaesthesia	2 (3)	3 (4)	1 (1)
Anti-inflammatory	2 (3)	2 (4)	1 (6)
Multi-modal therapy	2 (2)	2 (4)	1 (1)
Total	*9 (12)*	*9 (22)*	*6 (17)*

Effect of castration on cortisol concentration. Non-surgical castration: The overall mean difference reported in eight studies (n = 20 trials) was 0.108 nom/L (95% CI -0.305, 0.522), suggesting no evidence of changes and high heterogeneity between studies (I^2 = 80.2%; P = 0.000). No significant difference and high heterogeneity among studies was found between animals non-castrated and castrated without medicament at 30 min and 120 min. The multi-modal therapy (n = 2 trials) yielded a non-significant decrease in cortisol concentration 30 min after procedure and a low heterogeneity across studies (I^2 = 36.2%).

Surgical castration: Combining data from eight studies (n = 30 trials) gave a MD of 0.122 nmol/L (95% CI -0.104, 0.349) with low heterogeneity between studies (I^2 = 28.2%; P =

0.077). Compared to uncastrated, the surgical procedure without pain mitigation had no effect on cortisol concentration at 30 min (n = 4 trials) and no heterogeneity across studies, as well as at 120 min (n = 6 trials) with low heterogeneity among studies (I^2 = 25.9%). Cortisol concentration at 120 min after surgical procedure with anaesthesia tended to be lower (MD = -0.411 nom/L; P = 0.077; 95% CI -0.868, 0.045) than in the group castrated without medicament and showed no heterogeneity between studies (n = 5 trials).

Non-surgical vs. Surgical castration: When looking at all of the studies together (n = 17 trials), there was no consistent evidence of an overall effect on the cortisol concentration (MD = 0.080; 95% CI -0.153, 0.314) and low heterogeneity across studies (I^2 = 1.3%). Regardless of the time of cortisol measurement, the analyses showed low heterogeneity among studies and no strong evidence when in both groups, surgical or non-surgical, the castration was performed with no pain mitigation.

Effect of castration on average daily weight gain. Thirteen publications including 17 studies were included in the MA.

Non-surgical castration: Pooled results from 11 studies (n = 25 trials) evaluating non-surgical intervention showed a tendency to increase ADG of 0.403 g/d (P = 0.072; 95% CI -0.035, 0.842), but with high heterogeneity between studies (I^2 = 91.2%, P = 0.000) compared to control group. Results from 12 trials (n = 8 studies) showed an MD of 0.883 g/d (P = 0.002; 95% CI 0.313, 1.453) favouring non-surgical castration without medicament when compared with non-castrated group, with high heterogeneity among studies (I^2 = 83.6%). We found that the use of anaesthesia and multi-modal therapy had no effect on ADG, in spite of high heterogeneity between studies (I^2 = 90.1% and I^2 = 75.2%, respectively), in comparison to uncastrated cattle.

Surgical castration: The pooled analysis across 11 studies (n = 42 trials) showed no evidence of a significant difference (MD = 0.126; 95% CI -0.056, 0.308) between surgical

procedure and control group and moderate heterogeneity among studies (I^2 = 61%; P = 0.000). Ten of the individual trials (n = 8 studies) reported an increase in ADG (MD = 0.231; 95% CI 0.056, 0.405; P = 0.010) in the castrated group with no pain mitigation compared to the uncastrated and low heterogeneity between studies (I^2 = 12.9%) (Fig. 2). Although no difference in ADG was found between the groups, the heterogeneity among studies was low by the use of anti-inflammatory (I^2 = 12.3%) and multi-modal therapy (I^2 = 5.6%), and high by the use of anaesthesia (I^2 = 81.5%).

Non-surgical vs. Surgical: The comparison between non-surgical and surgical castration was reported in nine studies (n = 22 trials). Evidence showed a considerable heterogeneity among studies (I^2 = 58.6%). In the different treatment group analyses, we also found non-significant effect and moderate heterogeneity between studies for the no use of medicament (I^2 = 50.3%), while for the use of anaesthesia (I^2 = 75.1%), anti-inflammatory (I^2 = 72.2%), and multi-modal therapy (I^2 = 68%) in surgical group the heterogeneity among studies was high.

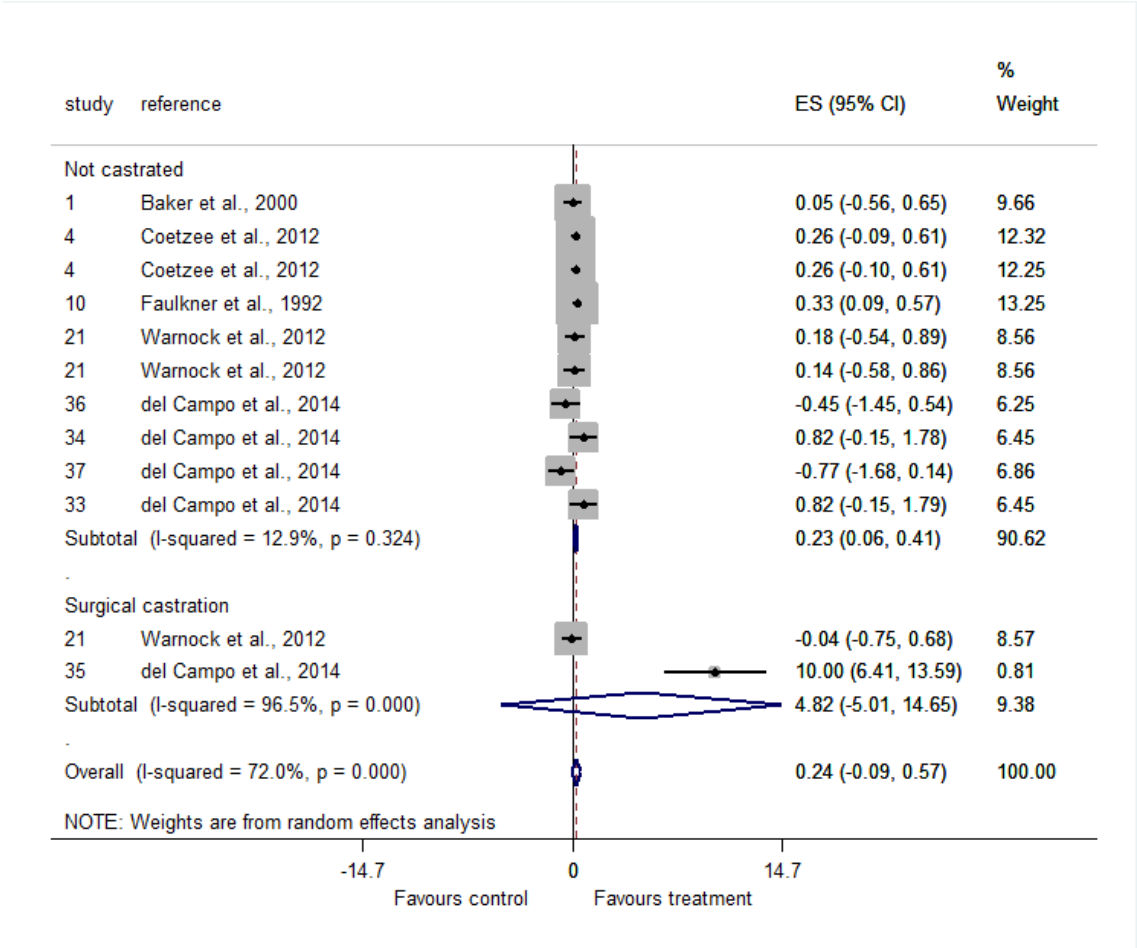

Figure 2. Forest plot of studies that analysed the effect of surgical castration with no pain mitigation (treated group) in comparison with not-castrated or surgical castration without pain mitigation (control groups). The effect size (ES) is the mean difference between control and treated groups, expressed in ADG (g/d). *Note:* The center of the square represents the point estimate for that study and the area of the square is proportional to the weight assigned to that study. The dashed line is the average effect of treatment obtained by the analysis, whereas the solid vertical line marks the value at which the treatment would have no effect. The diamond (♦) at the bottom of the dashed line shows the 95% CI for the overall effect obtained from then DerSimonian and Laird method.

Publication bias

There was some evidence of publication bias in studies using cortisol concentration. Although Egger's test showed a non-significant bias (P = 0.125), and suggests that small studies overestimate the effect (bias = 1.61), the Begg's test was marginally significant (P = 0.073) - and a visual inspection of the funnel plot suggested that publication bias might have been present. The random-effects "trim-and-fill" method reduced the combined pooled estimate from 0.114 to -0.093 (95% CI -0.285, 0.099), and indicated that additional 13 trials would have been necessary to remove this apparent publication bias (or other small-study effects) (Fig. 3).

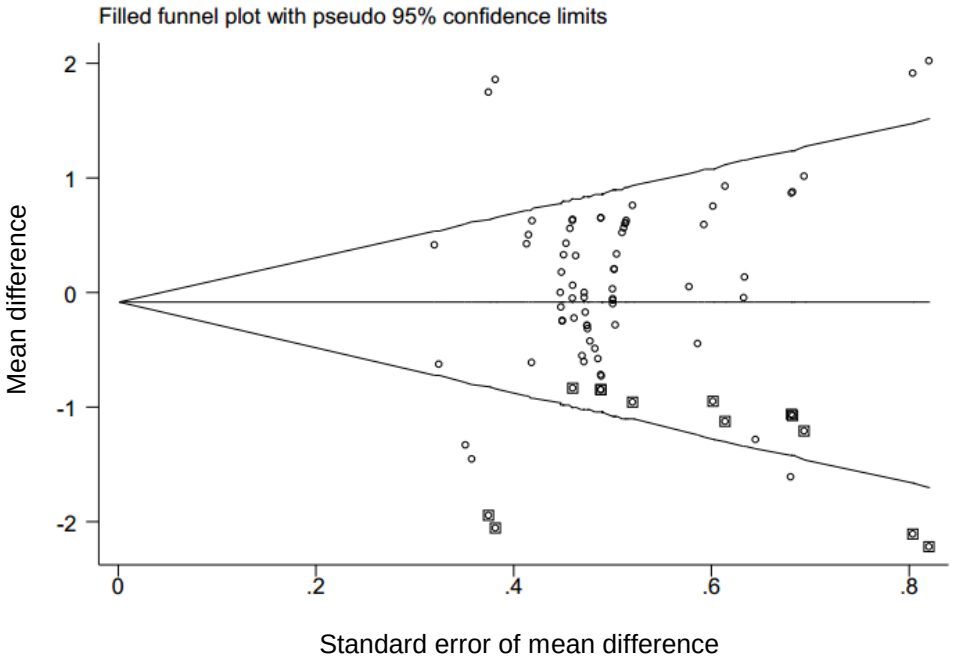

Figure 3. Funnel plot obtained with the Duval and Tweedie's "trim-and-fill" linear random effect model measuring standard mean difference in cortisol concentration as an outcome. The circles represent the original point estimate for each study (MD) and the circles encased in a square represent the studies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

Meta-regression analysis

Twenty-three studies (*n* = 156 trials) were included in the meta-regression analyses.

Meta-regression results for cortisol. None of the variables substantially reduced the variation between studies. However, two of them (control of confounders, and peer-reviewed) were significantly associated with the trial effect. Univariable meta-regression indicated that those studies reporting controlling for confounders had a predicted mean difference in cortisol's 0.50 nmol/L lower than studies that did not report control for confounders (*P* = 0.045). Meta-regression analyses also suggested that studies published in a non peer-reviewed journal (including conference proceedings, thesis, and government or research station report) had marginally lower predicted value (*MD* = -0.34; *P* = 0.054) compared to studies published in indexed and scientific journals.

Meta-regression results for average daily weight gain. Only publication type was significantly associated with ADG (*P* = 0.0019) and explained 18.52% of the total variance. Studies published in conference proceedings tended to report greater ADG (*MD* = 2.76; *P* = 0.000) when compared to results from studies published on peer-reviewed and scientific journals.

Cumulative meta-analysis and Influential studies

In the cumulative meta-analysis for cortisol concentration, there was clear evidence of change in the estimate point of the pooled treatments MD from negative (*MD* = -0.012) to positive (*MD* = 0.114), since 2009 until 2014. The sensitivity analysis showed that removing two studies (Coetzee et al., 2010; del Campo et al., 2014) from the analysis decreased and increased the MD from 0.114 nmol/L to 0.069 nmol/L and 0.161 nmol/L, respectively. Besides, another study (Martí, 2012) changed the I^2 from 56.7% to 14.7%.

No evidence on the trend of time effect was found for the ADG outcome. However, the pooled estimate for the impact of castration on ADG showed a reduction from 0.180 g/d to 0.110 g/d (P = 0.181; 95% CI -0.051, 0.270) by removing one study (Whitlock et al., 2013).

DISCUSSION

Literature search

Despite the relevance of this topic for the beef industry, the number of studies providing data to allow a broad quantitative analysis was lower than expected. Also, there was variable risk of bias for the outcomes of the included studies. Although reporting guidelines for randomized controlled trials have been published recently (Sargeant et al., 2005), we have detected failures to report sample size justification, random sequence generation, and blinding.

Most publications suitable to be included in the meta-analysis were published in the 2000s. The development of methods of recognition, assessment, and management of animal pain has been delayed. Also, in the 2000s, the proper management of pain in food-producing animals became a matter of increasing public concern and growing interest by legislation worldwide (Weary et al., 2006), together with reported emergence of castration as a painful procedure. Among the 18 publications, only one was non-English and more than a half were conducted in North America, given that the USA is the biggest beef producer. This aspect might be indicative of some bias due to continent and language, but does not preclude the possibility that researchers from non-English countries would also publish their studies.

The effect of castration on vocalization

A major advantage of behaviour is that it is immediately seen, allowing speedy assessment (Mellor et al., 2000). However, there is a relatively limited understanding of how to best

measure pain using behavioural indicators, as well as inter- and intra-oberver reliability and the pain specific-behavioural responses limit the usefulness of a measure (Weary et al., 2006; Reppening et al., 2013). In spite of this, Coetzee et al. (2008) showed correlation between vocalization and physiological measures of stress.

Although authors reported vocalization measure, quantitative synthesis approaches were not suited. The described methods were not validated among researchers and there were not enough captured studies in this review. Then, it is only when vocalizations are carefully validated that there is a potential for them to be used as diagnostic indicators of welfare (Olsson et al., 2011).

Our summarized results show that the mean score is lower in the non-castrated animals or in castrated group with anaesthesia. Schwartzkopf-Genswein et al. (2005) found that the vocalizing were greater during castration than in sham castrated animals. However, several studies showed no significant difference in that indicator between treatments (Coetzee et al., 2010; Petherick, 2012). Furthermore, a number of behaviours were influenced by cattle age, as well as by separation of calves from cows (Petherick, 2012).

Moreover, in the way that vocalization was measured, the potential for detection bias was high. This suggests that larger, well-reported field studies are needed to further evaluate this outcome as an indicator to measure pain and it seems inappropriate to draw conferences from these estimate point.

The effect of castration on cortisol concentration

Acute pain is a known activator of the hypothalamic-pituitary-adrenal axis. Therefore, changes in cortisol concentration appear to be particularly useful, despite the value of monitoring being limited by the difficulty of measuring the system's response, as well as the interanimal variations in the stress response (Mellor et al., 2000; Moberg, 2000). As this

variability rises our capacity to detect differences among groups decreases and greater numbers of animals are required (Mellor et al., 2000). Our data showed variability and a small sample size (mean = 33.5; minimum = 10; maximum = 60) in the included SR-MA studies.

Even though cortisol response has been widely used to assess well-being in farm animals, comparisons between reports can be difficult due to the circadian rhythm, differences in a way with that method is performed by different operator, and analytical and statistical methodology. Whereas Lefcourt et al. (1993) reported a diurnal rhythm, as well as a strong ultradian rhythm; Hudson et al. (1975) reported that there was no circadian rhythm of the endogenous cortisol secretion. Also, it has been supposed that low responses may be due to individuals with high pain threshold, or in which the physiological effect of castration was easily activated (Stafford and Mellor, 2005). However, the reason some cattle responded less than others in unclear.

The available evidence suggests that the surgical and non-surgical without pain medication did not increase cortisol levels as expected. One probable explanation is that the start of the cortisol measurement after intervention may also influence the interpretation. Nineteen bibliographic references were screened and analysed by Bretschneider (2005), who showed that castration caused a fast and maximum adrenal corticoid secretion 12 min after the surgical procedure. Second, although an evidence that sampling from the catheters with minimal non-aversive handling did not stress the animals (Schwartzkopf-Genswein et al., 2005), the majority of included studies analysed cortisol in blood by single samples. Third, there were no significant differences between control and castrated animals in physiological parameters (Coetzee et al., 2008; Petherick, 2012; del Campo et al., 2014). Cortisol concentration may also be increased in response to the stress handling alone, as an invasive method, and then it is difficult to distinguish between non-threatening stress and distress

(Moberg, 2000). As mentioned, the absence of variation in cortisol responses can be affected by animal's internal and external characteristics.

Furthermore, similar pattern of cortisol level in surgical vs. non-surgical castration was observed in our study, as well as by Petherick (2012) and del Campo et al. (2014), even though researchers found greater cortisol in surgical (Fell et al., 1986) or non-surgical castration (Petherick, 2011).. However, care is needed when interpreting as individual differences, cattle age and variations of a same method could influence the results (Stafford et al., 2002; del Campo et al., 2014). Also, the "ceiling effect" on cortisol responses leading to underestimates of the negative effects of the more invasive treatments (Mellor et al., 2000). As concluded by Stafford et al. (2002), all methods cause an immediate and significant rise in cortisol concentration.

No effect of multi-modal therapy in decreasing cortisol concentration during non-surgical castration in the first 30 min was observed. Here we might consider that the control groups were different, with one maintained intact and another submitted to non-surgical castration (ring castration), as well as the strategy used to mitigate the pain (analgesic-sedative and anti-inflammatory or anaesthesia) and the administration route. Both studies used saliva sample to measure the cortisol concentration, in spite of salivary cortisol may be ineffective as an indicator of immediate and chronic pain (González et al., 2010). Moreover, the optimum balance of analgesic efficacy can be achieved by the combination of anaesthesia with anti-inflammatory (Coetzee, 2011).

On the other hand, 120 min after intervention, anaesthesia tended to reduce cortisol level in animals surgically castrated. Many researchers found that anaesthesia can attenuated serum cortisol response (Stafford et al., 2002; del Campo et al., 2014), with no difference in the integrated cortisol response during 60-150 min post-castration (Coetzee et al., 2010). A

practical and affordable option for incorporation into farm management, a spray-on topical anaesthetic, can ameliorate pain up to 24 h (Lomax and Windsor, 2013).

Publication bias in the literature is likely to be reflected in the MA process (Borenstein et al., 2009). In this case, visual assessment and adjusted rank correlation test indicated some evidence of the presence of some bias. In our opinion, funnel-plot asymmetries may also have been result from clinical heterogeneity among studies (e.g. poor methodological design) (Lean et al., 2009). Inadequate quality of primary research has also been reported to yielded larger effects (Egger et al., 2001). Meta-regression analysis suggested that studies from non peer-reviewed or without control of confounders changes the cortisol response. Besides, the "trim-and-fill" test reduced MD and imputed studies.

The distinct pattern in results observed in the cumulative meta-analysis might be related to the public concern about the welfare of farm animals. The change in the effect can be result of the increase in the interest in pain caused by routine husbandry practices (Stafford and Mellor, 2005), as well as the improvement in study quality.

The average effect and the heterogeneity changed after the removal of two and one study, respectively. One of those (Coetzee et al., 2010) was the only one in which there was no clustering presented or group hierarchy, but the data was not collapsed. It also had a relatively small sample size (n = 22 animals) and the precision of the estimate was high and obtained directly in the graph. The other study (del Campo et al., 2014) was conducted in South America (Uruguay) and the animals were the youngest (7 days of age) in this MA, as well as the intervention protocol was not described in sufficient detail. The heterogeneity was reduced in 40% by removing Martí (2012), the unique publication in thesis format and analysed cortisol concentration of the hormonal castration in saliva sample.

The results of this MA complement and extend previous researchers that describe the effect of castration in cortisol levels. However, results described in the literature are conflicting and additional studies should be performed to corroborate this subject.

The effect of castration on average daily weight gain

Production parameters may not reflect the pain experienced by cattle (Stafford and Mellor, 2005), as it cannot reflect what is happening to the animal at the moment, but what was happening between the successive observations (Weary et al., 2006). In addition, the lower body weight gain in castrated male was possibly due to the decrease of testosterone (Fisher et al., 2001; Pang et al., 2008). However, assessment of these parameters is critical if research on animal welfare is to have relevance to livestock producers (Coetzee et al., 2011).

The effect of castration in ADG had the largest number of trials and showed consistently changes and consistent heterogeneity. A single study was responsible for reducing the effect and the heterogeneity and producing a non-significant change in ADG after castration. This influential study was published in conference proceedings (Whitlock et al., 2013), variable that contributed to the variation in ADG and explaining almost 20% of the total variance. The dissemination of research findings follows a continuum from oral report to full publication in an indexed and accessible journal, and then incomplete data can be published in a given step. Moreover, it has been described that the abstract format does not allow presentation of methodology or other; as well as one cannot rely on contact with authors (Egger et al., 2001), factors that hampered a more precise analysis.

The differences in performance between bulls and castrated male are mainly manifested after puberty, which is attained at an average age of 10 months (Barber and Almquist, 1975; Lunstra et al., 1978), with testosterone concentration peaked at 15 months of age (Gerrard et al., 1987). Field (1971) concluded after literature review that bulls gained 17% faster and

were 13% more efficient in converting feed in live weight than steers. Then, the decrease in ADG after castration, what mainly happens in the first 2 weeks (Pang et al., 2008; Warnock et al., 2012) and reduce the growth rate (Knight et al., 2000; Fisher et al., 2001; González et al., 2010), can be attenuated 28 (Coetzee et al., 2012), 30 (Knight et al., 2000) and 42 days post-castration (Warnock et al., 2012).

However, differences in growth performance favouring non-castrated group were not obtained in this MA and the comparison of performance indicators among studies and their interpretation it is not easy. Inadequate nutrition (Bailey and Hironaka, 1968; Martin et al., 1978), as well as the more aggressive behaviour (Martí, 2012), can prevent the bulls for expressing their greater potential for gain. Furthermore, can be related to the age at the time of intervention, hormonal status of the control group, castration method, the level of performance achieved, and the days of study (Pang et al., 2008; González et al., 2010; Martí, 2012).

Although del Campo et al. (2014) showed greater ADG for non-surgical than surgical castration, in our MA no strong evidence was detected when both were compared. Whether bull calves were banded or surgically castrated shortly after birth did not affect the weight at 217 days (Baker et al., 2000; Bretschneider, 2005), as well as on gain:feed when castration was performed after 7 months of age (Warnock et al., 2012; Reppening et al., 2013). The castration method may not be as important from a growth rate standpoint in the long term, indicating that cattle were able to compensate and recover from intervention regardless of the castration technique (Warnock et al., 2012; Pieler et al., 2013).

In agreement to our results, Newton and O'Connor (2013) showed that there was little evidence of castration's effect on ADG regardless of the type of pain management. Probably, the cattle that may have suffered distress after the medication effect was gone or experienced permanent changes in social status that lead to permanent changes in behaviour. Then, it

would be interesting to know the extent of effects of pain mitigation, i.e. the route of administration, period of exposure, and optimum dose (González et al., 2010; Coetzee et al., 2012).

The present meta-analysis has limitations. First, the approach to reporting outcomes often limited our ability to summarize the data, as there was incomplete reporting of summary measures. An attempt was made by contacting scientist in the field, as suggested by Lean et al. (2009). Second, we had to exclude six full-text articles about castration that passed abstract screening as we were unable to translate them, although we had no exclusion criteria based on language during the screening step. These included articles in German, Japanese, and Bulgarian, which might have introduced language bias, because negative findings are published in local journals, i.e. in languages other than English (Egger et al., 2001). Finally, in the absence of robust and specific direct and indirect measures associated with pain, the choice of parameters about welfare and its relationship with castration may be difficult.

In summary, this is the first SR-MA that summarized the available literature about effects of castration on welfare indicators in beef cattle. Regardless of the method, castration is a painful procedure. Evidences showed marginal support for mitigating pain during and after intervention, which might be due to the insufficient dose or time period for drug effect. The acute stress can be eliminated by anaesthesia during the first 120 min. There are substantial opportunities to hold several researches in the area, such as to validate behavioural indicators and less invasive physiological measures, as well as developing cost-effectively pain mitigation strategies. Lastly, the challenge in animal science experiment is to provide complete and accurate details of the methodology used in the trials in the publication by using standardized guidelines.

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APPENDIX 1: POPULATION, INTERVENTION, AND OUTCOME SEARCH TERM STRINGS USED FOR THE FINAL SEARCH IN THE SYSTEMATIC REVIEW

Acronym	Search string
Population	Beef cattle: are the young of domestic cattle, until twelve months (one year), to produce beef.
Intervention	Castration: is any action - physical, chemical or hormonal – to remove, destroy or decrease the activity of the testicles. Dehorning: means the amputation of horns at any stage after their growth the progressed beyond the early budding stage. Disbudding: refers to prevention of horn growth before it has become advanced.
Outcome	Vocalization: it is a behavior measure, which elicits help or stops other animals. Cortisol: it is a biological response to stress. Changes in plasma cortisol concentration appear to be particularly useful as an index of acute distress. Average daily gain: as a production indicator.

APPENDIX 2. LIST OF RELEVANT STUDIES EXCLUDED FROM THE FINAL DATASET, BECAUSE THEY DID NOT MEET ONE OR MORE OF THE MINIMUM REPORTING REQUIREMENTS FOR INCLUSION IN THE SYSTEMATIC REVIEW-META-ANALYSIS

Reference	Country	Treatment	Analgesic regimen	Outcome parameter	Reason for exclusion
King et al., 1991	Canada	Surgical and non-surgical	NA[1]	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	Insufficient data for this study
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

[1]NA: not applicable.

APPENDIX 3. SUMMARY OF ASSESSMENT FOR METHODOLOGICAL SOUNDNESS AND/OR REPORTING OF 18 PUBLICATIONS REPORTING 23 STUDIES INCLUDING IN THIS REVIEW

		Number of publications (studies)		
Variable	**Assessment**	**ADG**	**Cortisol**	**Vocalization**
Was the sample size justified?	Yes	2 (2)	1 (1)	0 (0)
	No	11 (15)	9 (11)	2 (2)
How were calves assigned to treatment groups?	Random[1]	0 (0)	2 (2)	1 (1)
	Reported random[2]	9 (9)	4 (4)	1 (1)
	Systematic[3]	1 (1)	0 (0)	0 (0)
	Convenience or unreported[4]	3 (7)	4 (6)	0 (0)
Was the intervention protocol described in sufficient detail to be replicated?	Yes	13 (13)	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	Yes	0 (0)	2 (2)	0 (0)

evaluate the outcome?				
	No	13 (17)	8 (10)	2 (2)
Based on the study design was clustering[5] accounted for appropriately in the analysis?	Yes	10 (12)	8 (10)	1 (1)
	No	0 (0)	1 (1)	1 (1)
	Not applicable	0 (0)	1 (1)	0 (0)
Were identified confounders controlled for or tested?	Yes, analysis[6]	8 (8)	7 (7)	2 (2)
	Yes, inclusion/exclusion[7]	2 (2)	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 described adequately so it can be reproduced?	Yes	13 (17)	9 (11)	2 (2)
	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)

[1] Computer or random number table, *a priori*, stratified random sample, cluster random sample.
[2] Author(s) report random, but randomization is not described.
[3] "n" samples obtained at x intervals or stratified by certain characteristics.
[4] Author indicated convenience sampling or sampling was not reported in the paper.
[5] Clustering was evaluated when repeated measures were reported.
[6] Author identified confounders and controlled for them in the analysis.
[7] Confounders were identified and included/excluded a priori.
[8] Confounders were controlled a priori by matching on certain characteristics.
[9] No adjustments were made for confounders/effect modifiers, etc., that were identified by the author.
[10] Confounders were not identified by the author or randomization was used to control for confounders.

CAPÍTULO III[1]

“Si nada nos salva de la muerte, al menos que el amor nos salve da la vida”.
(Pablo Neruda)

[1] Manuscrito elaborado conforme as normas da Animal Production Science (Apêndice 4).

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

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Welfare and dehorning in cattle: a meta-analysis

Abstract. Dehorning cattle is a commonly performed practice. Researchers suggest that pain can be reduced, but the evidences are inconclusive. We investigated the effects of dehorning on welfare indicators (cortisol concentration or average daily weight gain (ADG) or vocalization) in beef cattle until 12 months of age. We systematically searched on 5 electronic databases, and conference proceedings, as well as we contacted experts. Random effect meta-analyses (MA) were conducted for each indicator separately with the mean of control and treated group. A total of 4 prospective publications reporting 7 studies and 69 trials were included in the MA involving 287 cattle. Significant heterogeneity between studies was observed for each outcome. Cortisol concentration showed a decrease of 0.767 and 0.680 nmol/L, 30 (P = 0.000) and 120 (P = 0.023) min after intervention, respectively, when non-dehorned was compared to amputation dehorning group. Local anaesthesia reduced increase in cortisol concentration 30 min after dehorned by amputation. Non-dehorned animals had a marginal significant (P = 0.081) decrease in the number of vocalization than in dehorned by amputation. Publication bias was observed for results when ADG was studied as an outcome. The continent where the study was performed, sample size, blinding the outcome assessment, type of medicine used, and the publication type explained 95% of the between-study variance, and showed an association with the result, when the outcome was cortisol. Pain management in the routine dehorning of cattle is recommended. However, further investigations are required to improve confident decision in pain relief.

Additional keywords: animal welfare, cortisol, pain, performance, vocalization

Introduction

Horn buds are normally removed to reduce the risk of injuries to humans and other animals in the herd that can be caused by horns (Faulkner and Weary 2000; Stafford and Mellor 2005). Dehorning, regardless of the method used, is a pain-induced

response, which may be alleviated by applying strategies which alter the perception of pain or decrease the transmission of impulse in pain nerves from the wound (Sylvester *et al.* 1998*b*). Moreover, as many beef cattle are polled, breeding for polled cattle should be encouraged to eliminate the need to disbud or dehorn and the pain those procedures cause (Stafford and Mellor 2011).

Recently, the well-being of calves undergoing the dehorning procedure has been of great concern (Schwartzkopf-Genswein *et al.* 2005; Doherty *et al.* 2007). As a consequence, the literature focusing on pain management in cattle during dehorning is plentiful (Stock *et al.* 2013). With that, the current state of knowledge about dehorning procedures and pain alleviation, as well as highlights areas where there is insufficient evidence and need for future research, have been discussed in a subjectively manner by traditional reviews (Stafford and Mellor 2005; 2011). The systematic review (SR) provides a rigorous and replicable method to increase the credibility to findings in the studied field. Depending on the quantity, diversity, methodological soundness and reporting of the primary studies, meta-analysis (MA) could be a subset of SR. Meta-analysis refers to the statistical synthesis of results from a complex, sometimes apparently conflicting, body of literature, but it can never prevent biases (Egger *et al.* 2001; Borenstein *et al.* 2009)

The pain-induced distress caused by different methods of dehorning has been evaluated using physiological, behavioural and production responses before, during and after procedure (Stafford and Mellor 2005); as the results are inconclusive, we conducted a SR-MA to test the hypothesis that, despite dehorning is a painful procedure, strategies recognized could be used to prevent or minimize the adverse impacts on the welfare of cattle. This study was undertaken to identify, evaluate, critically appraise and synthesize available literature reporting how dehorning affect the behavioural and non-behavioural indicators of beef cattle.

Material and methods

Systematic review management

Research question and protocols. This SR identified the effects of dehorning procedure on beef cattle welfare by measuring cortisol concentration or average daily weight gain (ADG) or vocalization.

The review question was defined based on key concepts in terms of PICO format: population (P), intervention (I), comparator (C), and outcome (O). The study population was beef calves until 12 months of age. Studies were included if the interventions were castration, dehorning, and disbudding, while in a following stage the analysis concerns castration procedure. Outcomes of interest were vocalization; cortisol; and ADG (Table 1). We did not exclude studies based on the type of comparison used.

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

Acronym	Search string
Population	Beef calves: are the young of domestic cattle, until twelve months of age (one year), to produce beef.
Intervention	Castration: is any action - physical, chemical or hormonal – to remove, destroy or decrease the activity of the testicles. Dehorning: means the amputation of horns at any stage after their growth the progressed beyond the early budding stage. Disbudding: refers to prevention of horn growth before it has become advanced.
Outcome	Vocalization: it is a behavior measure, which elicits help or stops other animals. Cortisol: it is a biological response to stress. Changes in plasma cortisol

concentration appear to be particularly useful as an index of acute distress.

Average daily weight gain: as a performance indicator.

The research team collapsed methods into three dehorning groups: 1) amputation using scoop dehorners, such as Barnes, Keystone, knife, and cup (plus cautery iron); 2) cautery using a hot iron (electric or thermal); and 3) amputation *vs.* cautery dehorning. Also, relevant pain reliefs were identified as anaesthesia (lidocaine, procaine, and Tri-Solfen®), anti-inflammatory (meloxicam), and multi-modal therapy (combination of flunixin and procaine, and lidocaine and meloxicam).

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

Search methods for identification of studies. A list of final search terms and algorithms was summarized by population, outcome and intervention components of the question as follow: (bovine OR "beef cattle" OR cal* OR herd) AND (disbud* OR dehorn* OR castration) AND (“animal wel*" OR "animal pain" OR "animal stress" OR cortisol OR behavio* OR vocali*). These search strategy also retrieved relevant studies evaluating animal performance as the outcome, therefore “average daily weight gain” was not included to avoid the overload of non-relevant citations.

A systematic literature search was conducted on five electronic databases – CAB Abstracts (Thomson Reuters, 1910–2015), ISI Web of Science (Thomson Reuters, 1900–2015), PubMed (1940–2015), Agricola (EBSCO, 1970–2015) and Scopus (Elsevier, 1960–2015) – and were searched on May 2013 and updated on May 2015. In addition, the following proceedings were searched for references: ADSA-ASAS Joint Annual Meeting (from 2001 to 2014) and International Society for Applied Ethology, ISAE (from 2001 to 2014). We also contacted investigators to request unpublished

data. Reference search verification was performed by searching the reference lists from four recent literature reviews (Stafford and Mellor, 2005; Weary *et al.* 2006; Stafford and Mellor, 2011; Schwartzkopf-Genswein *et al.* 2012).

All citations were imported into the reference manager Refworks (RefWorks–COS, USA) and duplicate citations were removed manually.

Study selection criteria and relevance screening. A total of five reviewers contributed to the different levels of the review process and were trained for the relevance screening step using 30 abstracts during pre-test.

The search strategy was conducted to identify primary research, which reported animal welfare in beef cattle; castration, dehorning, or disbudding as an interventions; and measure cortisol, ADG, or vocalization as welfare indicators.

The included study designs were randomized and non-randomized clinical trials, cohort studies, and case-controls. At this stage, no limits were applied for language or year.

All identified citations were independently assessed for relevance by two independent reviewers using the titles and abstracts (when available) and conflicts were resolved with consultation. When agreement was not attain, an expert opinion was requested.

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

Methodological assessment and data collection process. Data extraction forms were adapted and piloted on 102 publications. The first author was responsible for extracted data from the eligible studies. Publications reporting more than one study design were duplicated and extracted as separate studies.

Before risk of bias assessment and data extraction (DE) were undertaken, the relevance of papers selected through abstract screening was confirmed using the full papers based on language (English, Spanish, Portuguese, or Italian); appropriate

control group; and sufficient detail to report the results to conduct the DE and to extract quantitative data to perform a MA. At this stage, primary research was restricted to publications in the languages that the research team members were fluent, and translation of articles published in other languages was precluded due to financial constraints.

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

Considerations for data collection and data manipulations. For the outcomes, we attempted to extract the mean, standard deviation (SD) or any available measure of dispersion, measurement unit, *P*-value, and the number of animals in control and treatment groups. Data from cortisol and ADG were converted to nmol/L and g/day, respectively. In the database, count of vocalizations was continuously recorded from the start to completion the procedure. These summary measures were entered into an electronic spreadsheet and a dataset was built containing the results for controlled studies, measuring outcomes of interest: cortisol (baseline, 20 or 30 or 40 min, and 120 min), ADG (during observation period) or number of vocalization (during procedure). When the results were reported in the log-transformed scales, these were transformed back to the original scale using the formula described in Mederos *et al.* (2012). Whenever an overall standard error of the mean (SEMp) was reported for the control and treatment groups, a pooled standard deviation (Sp) was derived from the formula (1) (Ceballos *et al.* 2009):

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

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

For those studies that reported only *P*-values, an estimate of a common standard deviation was computed using the t-statistic, assuming the data were normally distributed, using the formula (Mederos *et al.* 2012):

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

where $x_2 - x_1$ represents the means difference; *t(αƒdE)* is the percentile from the reference distribution; and n is the sample size of each group.

When results were only graphically presented, the corresponding author was contacted by electronic mail and asked to provide the summary statistics. If no response was obtained or data were not provided, the mean and/or measure of dispersion were extracted by manual measurement using a ruler.

Finally, as the cortisol data were collected in those three different points, the summary data were recreated and then the effect size was computed according to recommended approaches (Borenstein *et al.* 2009).

Quality assessment

We used standardized methods to analyse the risk of bias of the individual studies included in the MA (Higgins and Green 2011), with one minor modification. To evaluate the domain “blinding of outcome assessment”, we considered that the behavioural outcome was at high risk of bias if blinding was not reported and at low risk of bias if blinding was reported, as described by Dzikamunhenga *et al.* (2014), once it is a subjective measure and more prone to poor reliability (Weary *et al.* 2006). Physiological and performance outcome were considered to be at low risk of bias regardless of the presence or absence of blinding.

Meta-analysis

Studies were included in the quantitative analysis when they reported quantitative necessary results to estimate a standardized mean difference (MD) between control and treatment groups and its 95% confidence interval. The data analysed for cortisol was from baseline to 20/30/40 min and to 120 min; for ADG, during observation period; and for vocalization, during intervention. All the analyses were performed in the statistical package Stata (V 14.0, StataCorp., Texas, USA).

Logarithmic transformations of the mean and SD for control and treatment groups prior to estimation of the pooled estimate mean and SD, were performed according to techniques for separate standard deviations proposed by Higgins *et al.* (2008).

The random effect MA and meta-regression were carried out given the *a priori* assumption that between-study heterogeneity was present. The DerSimonian and Laird method was used to estimate the between-trial variance.

Comparison groups meta-analysis. A separate MA was conducted using various subsets of data, consisting of at least two individual studies that investigated similar treatments and same outcome. Concurrently, each outcome was evaluated separately as a group using stratification by dehorning technique and pain management. A pooled MD and 95% CI were generated (forest plots). The Cochran's Q (a chi-squared test of heterogeneity) and I^2 (percentage of total variation between studies that is due to heterogeneity rather than chance) were calculated based on the dehorning technique and outcome. The magnitude of I^2 was interpreted in the order of 25%, 50%, and 75% which might be considered as low, moderate, or high heterogeneity (Higgins *et al.* 2003). Differences were considered significant at $P < 0.05$ and trends were defined at $0.05 \leq P < 0.1$.

Publication bias

Publication bias was visually and statistically assessed using a funnel plot and the Begg's adjusted rank correlation and Egger's regression asymmetry tests for each

outcome. Bias was considered based on visual plot and if at least one of the statistical methods was significant ($P < 0.10$). If there was any evidence of publication bias, the "trim-and-fill" method suggested by Duval and Tweedie (2000) was used to estimate the extent of the bias.

Meta-regression

Random-effects regression univariable models were performed to evaluate sources of between-study heterogeneity which may influence the response of subjects to treatment (Borenstein *et al.* 2009). Meta-regressions were carried out for trials reporting cortisol concentration and ADG as outcomes by using method-of-moments estimator.

The variables explored in the meta-regressions were (1) randomization (no or yes), (2) cluster (no, yes or not applicable), (3) confounders identified and controlled (no, yes or not applicable), (4) publication year, (5) publication type (peer-reviewed, conference proceedings, thesis, government or research stations reports), (6) continent (North America, South America, Europe, Asia or Oceania), (7) cattle group (*Bos taurus*, *Bos indicus*, hybrid/mixed or not reported), (8) cattle sex (not reported, female, male or mixed), (9) who performed the intervention (not reported, farm staff or veterinarian), (10) application of medicine for pain relief (no or yes), (11) type of medicine (not applicable, analgesic-sedative, anaesthesia, anti-inflammatory or multi-modal therapy), (12) dehorning technique (amputation, cautery or amputation vs. cautery), (13) cattle age, (14) intervention follow-up period, and (15) sample size.

Cumulative meta-analysis and Influential studies

A cumulative meta-analysis was conducted to evaluate the pooled estimate of the treatment effect each time the result of a potential new study is published. Those

analyses are most often used to display the pattern of the evidence over time, by sort the data chronologically (Borenstein *et al.* 2009).

Sensitivity analyses were performed to determine whether certain studies had substantial impact on the MD. This was performed by manually replacing and removing one study at a time and evaluating whether the mean difference had change by ±30%.

Results

Study selection and characteristic

Searching identified 1,248 publications. Of these, 102 were identified as useful review papers or reports likely to contain data, but only 33 eligible were included for methodological soundness and data extraction (Fig. 1). Seven studies provided extractable data to SR-MA (Table 2).

From three contacted authors who presented their results graphically or without sufficient data, none numerical data were obtained. Then, the data were manually extracted by using a ruler.

The alternative treatments evaluated in the review were amputation (n = 6 studies) and cautery (n = 2) dehorning. No quantitative analysis was done for amputation *vs.* cautery techniques, as only one study reached the data extraction stage. Relevant pain mitigation included four studies that analysed anaesthesia, one evaluating anti-inflammatory, and two evaluating multi-modal therapy.

The total number of cattle for the included studies that evaluated dehorning and cortisol concentration, ADG, and vocalization were 283, 131, and 139, respectively.

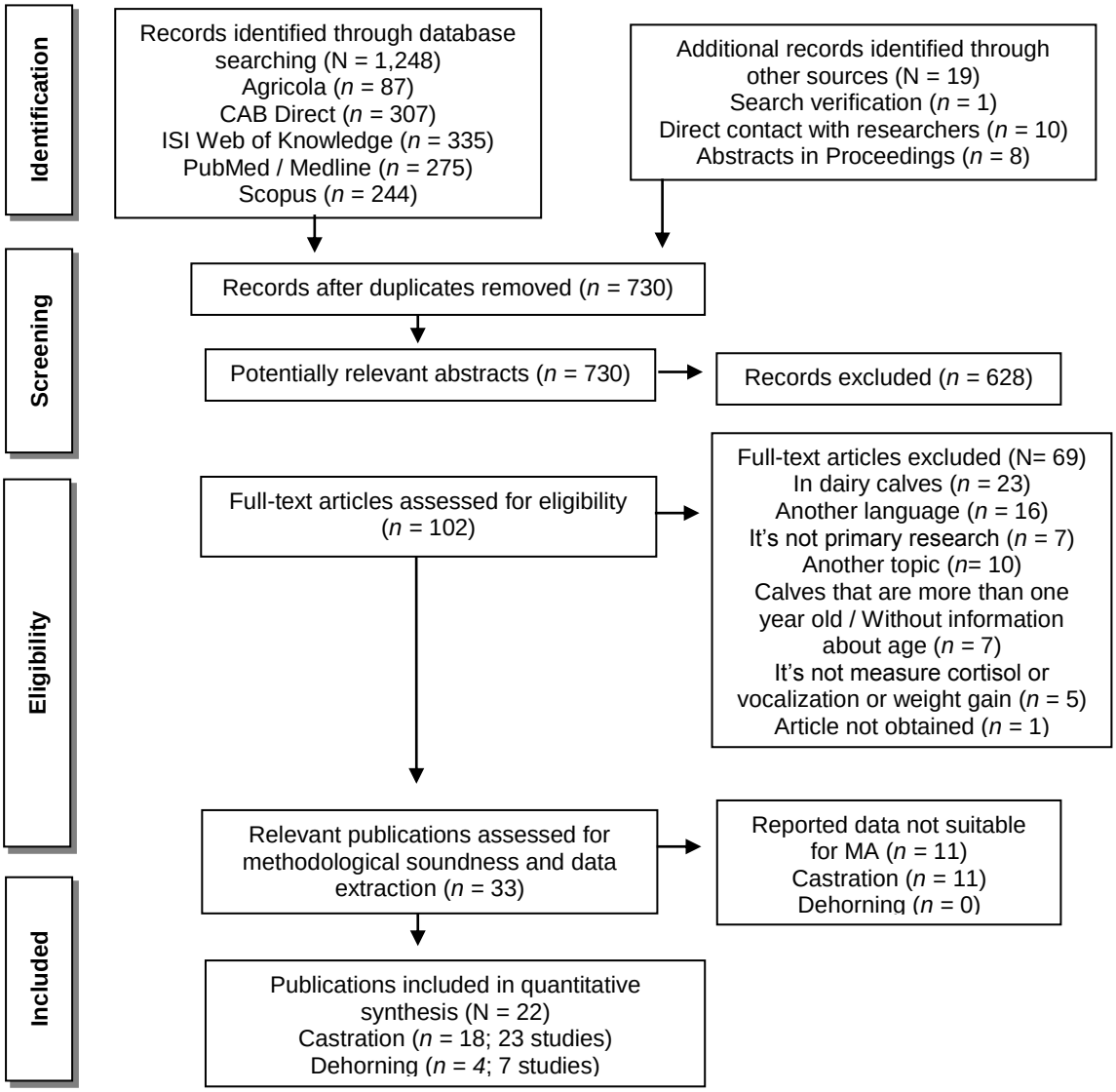

Fig. 1. Flow diagram indicating the data recording in systematic review with the number of abstracts and publications included and excluded in each level. MA: meta-analysis. Adapted from PRISMA guidelines (Moher *et al.* 2009).

Table 2. A descriptive summary of each relevant study that was included in the final systematic review and was used in the meta-analysis and meta-regression (7)

Reference	Country	Study population (age in days / sample size)	Procedure	Analgesic regimen	Outcome parameter
Cooper *et al.* 1995	Canada	180 / 12	Amputation dehorning	NA	Cortisol (30 min)
Mellor *et al.* 2002	New Zealand	70 / 30	Amputation dehorning	Local anaesthesia	Cortisol (30 and 120 min)
Sinclair 2012	Australia	217 / 56	Amputation dehorning	Anti-inflammatory and multi-modal therapy	Cortisol (30 and 120 min) ADG (56 days) Vocalization (during procedure)
Sinclair 2012	Australia	217 / 27	Amputation dehorning	Local anaesthesia	Cortisol (30 min) ADG (56 days)
Sinclair 2012	Australia	232 / 48	Amputation dehorning	Local anaesthesia	Cortisol (30 min)

					ADG (13 days) Vocalization (during procedure)
Sinclair 2012	Australia	120 / 35	Amputation and cautery dehorning	NA	Cortisol (30 min) Vocalization (during procedure)
Hubber *et al.* 2013	Austria	210 / 79	Cautery dehorning	Local anaesthesia and multi-modal therapy	Cortisol (30 and 120 min

ADG: average daily gain; NA: not applicable.

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

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

Variable	Description	Categories	Number of publications (studies)
Study design	Type of study design used	Control studies	4 (7)
Publication type	Type of literature the work was published	Peer-reviewed	3 (3)
		Conference proceedings	0 (0)
		Thesis	1 (4)
		Government or research station report	0 (0)
Treatment	Type of technique evaluated	Amputation dehorning	3 (6)
		Cautery dehorning	2 (2)
		Amputation *vs.* Cautery dehorning	1 (1)
Data published	Year of study publication	1990-2000	1 (1)
		2001-2015	3 (6)

Medicament	It was used any class of medicament?	No	3 (6)
		Yes	3 (5)
Medicament	If was used any medicament to mitigate pain, which class?	Anaesthesia	3 (4)
		Anti-inflammatory	1 (1)
		Multi-modal therapy	2 (2)
Gender	Gender in which the procedure was evaluated	Female	1 (3)
		Male	1 (1)
		Female and male	2 (2)
		Not reported	1 (1)
Cattle group	Cattle group in which intervention was evaluated	*Bos taurus taurus*	1 (1)
		Bos taurus indicus	0 (0)
		Hybrid / Mixed	2 (5)
		Not reported	1 (1)
Who performed	Who performed procedure	Farm staff	1 (3)
		Veterinarian	0 (0)
		Not reported	4 (4)
Outcome assessed	Parameter used to assess pain in calves	Average daily gain	1 (3)
		Cortisol	4 (7)
		Vocalization	1 (3)

Sample size	Size of total study population per study	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)

Risk of bias

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

Unclear risk of performance bias was identified in 100% of the studies which analysed vocalization and ADG, and in 83.1% about cortisol concentration. The approach to blinding of outcome assessor from knowledge of which intervention an animal received was not reported, making the risk of detection bias high for vocalization. Regardless of the outcome, all studies were found to have low risk of attrition bias.

Table 4. Methodological quality assessment for risk of bias (classified as low, unclear, and high) of the seven studies included in the systematic review of welfare in dehorned beef cattle

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

				Vocalization	Unclear	High	Low
Sinclair 2012	High	High	High	Cortisol	Unclear	Low	Low
				Vocalization	Unclear	High	Low
Hubber *et al.* 2013	Low	Low	Low	Cortisol	Low	Low	Low

ADG: average daily gain.

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

		Number of publications (studies)		
Variable	Assessment	ADG[A]	Cortisol	Vocalization
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[B]	0 (0)	1 (1)	0 (0)
	Reported random[C]	1 (3)	2 (4)	2 (2)
	Systematic[D]	0 (0)	0 (0)	0 (0)
	Convenience or unreported[E]	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[F] 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[G]	0 (0)	0 (0)	0 (0)
	Yes, inclusion/exclusion[H]	1 (3)	2 (5)	1 (3)
	Yes, matching[I]	0 (0)	0 (0)	0 (0)
	No[J]	0 (0)	1 (1)	0 (0)
	Not applicable[K]	0 (0)	1 (1)	0 (0)
Was the statistical analysis described adequately so it can be reproduced?	Yes	1 (3)	3 (6)	1 (3)
	No	0 (0)	1 (1)	0 (0)
	Reference paper	0 (0)	0 (0)	0 (0)
	Statistical analysis not done	0 (0)	0 (0)	0 (0)

[A] Average daily gain.

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

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

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

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

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

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

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

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

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

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

Meta-analysis

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

Table 6. Number of publications and number of controls studies used in meta-analysis and/or meta-regression, stratified by procedure, outcome, and the use of medicament

Medicament	Publication (studies)	Studies (trials) Average daily gain	Cortisol	Vocalization
		Amputation dehorning		
No	3 (6)	3 (5)	6 (12)	2 (4)
Yes	2 (4)	3 (10)	4 (19)	2 (6)
Anaesthesia	2 (3)	2 (5)	3 (9)	1 (3)
Anti-inflammatory	1 (1)	1 (1)	1 (4)	1 (1)
Multi-modal therapy	1 (1)	1 (4)	1 (6)	1 (2)
Total	*3 (6)*	*3 (15)*	*6 (31)*	*3 (10)*
		Cautery dehorning		
No	1 (1)	0 (0)	1 (1)	0 (0)
Yes	1 (1)	0 (0)	1 (12)	0 (0)
Anaesthesia	1 (1)	0 (0)	1 (2)	0 (0)
Anti-inflammatory	0 (0)	0 (0)	0 (0)	0 (0)

Multi-modal therapy	1 (1)	0 (0)	1 (10)	0 (0)
Total	*2 (2)*	*0 (0)*	*2 (13)*	*0 (0)*

Effect of dehorning on cortisol concentration. The cortisol concentration was the most commonly investigated outcome, and the seven included studies provided data for MA.

Amputation dehorning: Combining data from six studies (*n* = 31 trials) gave a MD of -0.219 nmol/L (95% CI -0.420, -0.049), suggesting significant changes (*P* = 0.032) favouring control group, and moderate heterogeneity between studies (I^2 = 41.2%; *P* = 0.010). Compared to no dehorned, the dehorned animals with no pain mitigation showed significant higher cortisol level at 30 min (*n* = 8 trials; *MD* = -0.767; 95% CI -1.099, -0.435; *P* = 0.000) (Fig. 2), as well as at 120 min (*n* = 2 trials; *MD* = -0.680; 95% CI -1.267, -0.093; *P* = 0.023) after intervention, with no heterogeneity among studies. In seven trials it was found no significant effect and 0% heterogeneity between studies in the dehorning with anaesthesia, regardless of the control group, 30 min after procedure.

Cautery dehorning: Pooled results from two studies (*n* = 13 trials) showed no evidence of changes on the overall effect of cortisol concentration and moderate heterogeneity among studies (I^2 = 58.6%; *P* = 0.004). Unfortunately, only one study was available for dehorning without pain mitigation, for anaesthesia, and for multi-modal therapy.

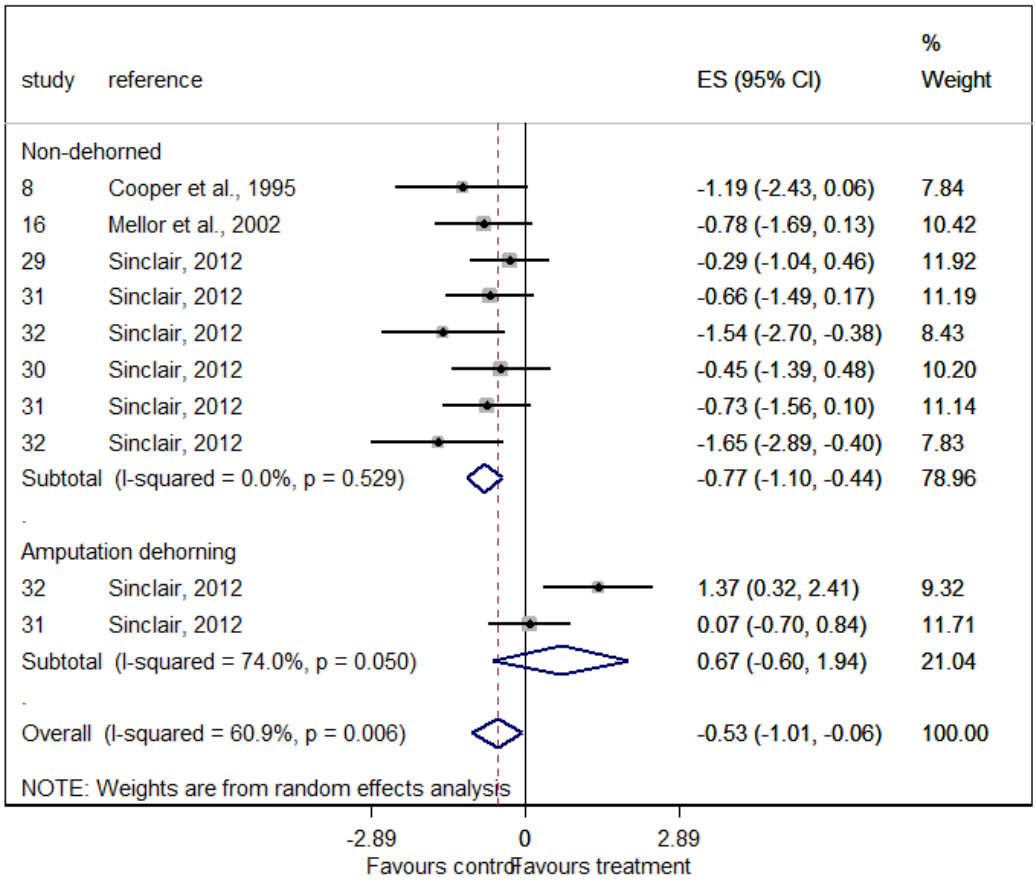

Fig. 2. Forest plot of studies that analysed the effect of amputation dehorning with any pain alleviation (treated group) in comparison to non-dehorned or dehorning by amputation without pain mitigation (control groups) 30 minutes after intervention. The effect size (ES) is the mean difference between control and treated groups, expressed in cortisol concentration (nmol/L). *Note:* The center of the square represents the point estimate for that study and the area of the square is proportional to the weight assigned to that study. 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 diamond (♦) at the bottom of the dashed line shows the 95% CI for the overall effect obtained from the DerSimonian and Laird method.

Effect of dehorning on ADG. Amputation dehorning: When looking at all of the studies together (*n* = 15 trials), there was a consistent evidence of an overall effect on the ADG (*MD* = 0.487; 95% CI 0.080, 0.895; *P* = 0.019) and high heterogeneity among studies (I^2 = 70.5%). Analysis of three studies (*n* = 4 trials) involving non-dehorning and dehorning with no pain medicament produced a combined MD of 0.800 g/day (95% CI -0.306, 1.907) with high heterogeneity between studies (I^2 = 83.8%). We found that the use of anaesthesia (*n* = 5 trials) had no effect in ADG, despite of high heterogeneity between studies.

Effect of dehorning on vocalization. Amputation dehorning: The overall mean difference reported in three studies (*n* = 10 trials) was -0.210 g/day (95% CI -0.972, 0.553), suggesting no evidence of changes and moderate heterogeneity among studies (I^2 = 37.2%; *P* = 0.111). The effect size in control group was -0.929 (*P* = 0.081; *n* = 4 trials) in comparison to dehorned animals, with low heterogeneity between studies (I^2 = 23.4%; *P* = 0.271). No significant difference (*MD* = -0.434; 95% CI -1.391, 0.524) and no heterogeneity among studies (*n* = 2 trials) were found between different methods of amputation dehorning without medicament.

Publication bias

The statistical approaches used for the evaluation of publication bias in studies reporting cortisol levels as an outcome produced different results. Although Egger's test indicated a significant bias (*P* = 0.024), the Begg's test did not suggest a significant bias (*P* = 0.132). The visual inspection of the funnel plot showed an asymmetry, with a gap in a bottom corner of the graph. However, using the random-effects "trim-and-fill" correction, no missing studies were imputed, suggesting a lack of evidence for publication bias.

Visual inspection of the funnel suggested asymmetry for the impact of dehorning on ADG. The adjusted rank correlation revealed a significant bias (*P* = 0.012), whereas

the Egger's test was non-significant and suggested that small studies overestimate the effect (bias = 2.84). The "trim-and-fill" method indicated that two additional studies have been necessary to balance the funnel plot, as show in Fig. 3.

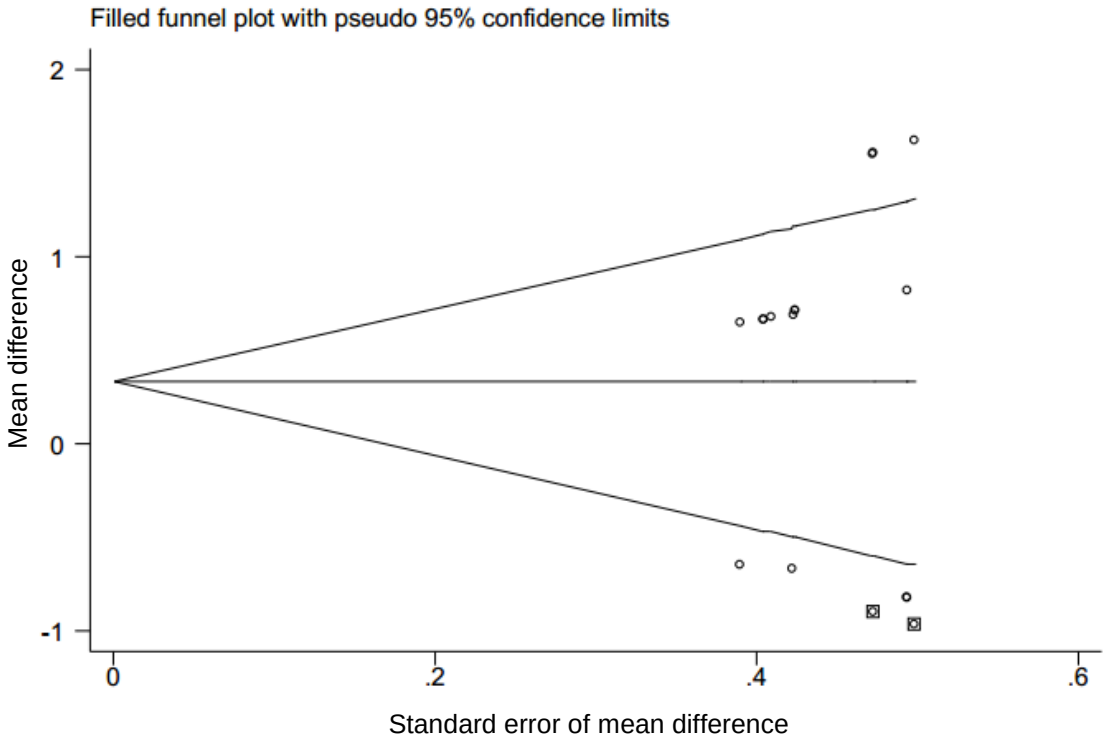

Fig. 3. Funnel plot obtained with the Duval and Tweedie's "trim-and-fill" linear random effect model measuring standard mean difference in cortisol concentration as an outcome. The circles represent the original point estimate for each study (MD) and the circles encased in a square represent the studies that the program imputed (*n* = 2) to create a symmetrical plot.

A systematic bias could not be detected by inspection of funnel plot, as well as by statistical Egger's and Begg's tests, when evaluating vocalization as an outcome.

Meta-regression analysis

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

Meta-regression results for cortisol. The five variables together, showed in Table 7, explained 95% of the total variance. Changes in cortisol concentration showed a direct association with the sample size. Only one variable related to study quality recorded in the database tended to show a significant association with the outcome of the interest. Cortisol levels in studies published in thesis tended to be lower than in those published in peer-reviewed journals. Studies evaluating dehorning with local anaesthesia or multi-modal therapy had a significant effect in change cortisol concentration compared to intervention with no pain mitigation.

Table 7. Results from univariate meta-regression showing significant (*P* < 0.05) and marginally significant (0.05 ≤ *P* < 0.1) covariates investigated as potentials sources of study heterogeneity. The results explained for each of the covariates included in the meta-analysis is presented for cortisol level 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			
Type of medicine			0.0185	46.28	31.50
Not applicable (n = 13)	Referent				
Anaesthesia (n = 11)	0.63	0.15, 1.11	0.011		
Anti-inflammatory (n = 4)	0.10	-0.55, 0.75			
Multi-modal therapy (n = 16)	0.59	0.17, 1.01	0.007		

I^2: between-study residual variation; Adj-R^2: percentage of the residual variation.

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

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

[C] Standard mean difference of the effect size.

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

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

Meta-regression results for ADG. None of the variables showed an association with ADG, nor contributed to explain the variation between studies.

Meta-regression results for vocalization. None of the 15 variables evaluated in meta-regression showed an effect on vocalization. However, application of medicine for pain relief and type of medicine reduce the variation among studies in 100% and 90.08%, respectively.

Cumulative meta-analysis and Influential studies

The random-effects cumulative meta-analysis of the studies of dehorning and its effects on cortisol concentration, in general, did not present a significant effect. However, pattern was observed through time. During the 1990s the trial had the highest treatment effect (*MD* = -1.186), which tended to decline during the 2000s (*MD* = -0.110).

All publications for the ADG and vocalization outcomes were published in 2012, then, we cannot perform the analysis.

The pooled estimate for the impact of dehorning on cortisol level showed a reduction from -0.110 nmol/L to -0.231 nmol/L by removing one study (Hubber *et al.* 2013); and an increase to -0.054 nmol/ by omitting one study of Sinclair (2012). Also, another study from Sinclair (2012) increased the MD to 0.071, as well as changed the I^2 from 54.1% to 36.2%.

Two studies which evaluated ADG as an outcome, published by Sinclair (2012), showed an increase in the MD from 0.487 g/day to 0.656 g/day and a reduction to 0.237 g/day.

The sensitivity analysis for vocalization's number during intervention showed that removing all the 3 studies (Sinclair2012) from the analysis changed the effect on the

pooled estimate and on the heterogeneity among studies. The largest effect was the increase on the MD from -0.065 to 0.504, and the reduction on the I^2 from 91% to 0%.

Discussion

Literature review

Pain management in cattle undergoing dehorning become a higher priority area on scientific investigations and practical solutions. Although a reasonably large body of works was available, a small number of publications were available to quantitative analysis.

From the seven studies providing data useful for MA, five were conducted on the continent of Oceania and six during 2000s. The public concern about pain caused by routine husbandry practices in farm animals has increased (Stafford and Mellor 2005). Subsequently, several countries including those in the Europe Union, Australia, and New Zealand, have been recreating the dehorning welfare codes (Stock *et al.* 2013). Moreover, during the last years, the pain-induced distress caused by dehorning and strategies to its alleviation have been investigated extensively (Stafford and Mellor 2005). The delay to develop methods of recognition and assessment of animal pain by the unwillingness to accept that animals are capable to suffer (Molony and Kent 1997) can explain the increase in publications in this century.

The effect of dehorning on cortisol concentration

Changes to the physiology of cattle following dehorning are frequently observed biomarkers in pain assessment (Schwartzkopf-Genswein *et al.* 2005; Stock *et al.* 2013). Cortisol levels represent only one feature of an animal's distress response, excluding for instance more rapid sympathetico-adreno medullary response. Caution is required when interpreting cortisol levels, because the difficulty of measure the hypothalamus-pituitary-adrenal system, as well as a wide variety of causes that can

activate the system's response. Furthermore, even though Stafford and Mellor (2005) reported that the individual responses were similar with small variances in most studies about dehorning, the interanimal variations in the stress response should be accounted (Mellor and Stafford, 1997; Molony and Kent 1997; Mellor *et al.* 2000). In spite of debate about the validity of using cortisol responses (Mellor and Stafford 1997), there are few effective physiological alternatives (Stafford and Mellor 2005).

The effect of dehorning in cortisol concentration showed heterogeneity. Two studies were responsible to change the effect and one to reduce the heterogeneity. Although those performing SR-MA must include searches of dissertations to assure comprehensive identification of all relevant studies (Egger *et al.* 2001), two influential studies were published in thesis (Sinclair 2012), factor that contributed to the variation in cortisol and explaining almost 15% of the total variance. The only study that used blinding of outcome assessment and had the largest sample size (n = 79 animals), variables which together contributed with more than 30% of the total variance and to the cortisol's response, was published by Huber *et al.* (2013), another influential study. Careful design, conduct, and analysis of a trial prevent the detection bias (Egger *et al.* 2001). As a consequence of the variations between animals, the stress response decreases our capacity to detect differences among groups and greater numbers of animals are required (Mellor *et al.* 2000). Mellor and Stafford (1997) suggested that with larger group numbers, the differences among treatments might have become significant.

In this study, the response of cortisol secretion to amputation dehorning with no pain relief was as expected. Several studies observed an increase in cortisol concentration in response to the intervention (Cooper *et al.* 1995; Mellor *et al.* 2002; Sinclair 2012), despite the fact that calf distress responses vary, both between and within each method (McMeekan *et al.* 1997). The qualitative nature of the distress caused by dehorning allowing two phases of cortisol response. The initial peak due to horn

amputation, peaking after about 30 min, is following by an inflammatory phase consisting of a plateau which persists for 5-6 h before returning to pre-treatment level (Cooper *et al.* 1995; McMeekan *et al.* 1998; Mellor *et al.* 2002). The comparison between four methods of mechanical dehorning conclude that the maximum cortisol secretion occur during the first hour (Sylvester *et al.* 1998a), with no difference in relation to the depth of the wound (McMeekan *et al.* 1997)

For this outcome, the expected result - local pain medication would decrease or eliminate cortisol response – was obtained in our SR-MA. In agreement, prior administration of local anaesthesia diminished the cortisol level exhibited by dehorned cattle during the first 2 (McMeekan *et al.* 1998; Mellor *et al.* 2002; Sinclair 2012) and 3 h (Sylvester *et al.* 1998*b*) after treatment to the levels of the handling of the calves, as well as there were significant differences in the integrated cortisol responses (McMeekan *et al.* 1998; Sinclair 2012). Noxious sensory input from the wounds persisted beyond the action of local anaesthetic, increasing the cortisol level after the effect wore off (Sylvester *et al.* 1998*b*). The administration of local anaesthetic in conjunction with an anti-inflammatory (McMeekan *et al.* 1998; Stilwell *et al.* 2012) or the combination of local anaesthetic and cauterising the dehorning wound (Sylvester *et al.* 1998*b*) virtually abolished the delayed cortisol response. Meanwhile, it is to be hoped that those medicaments become more freely available to farmers worldwide (Stafford and Mellor 2011).

However, a lack of effect on cortisol response between cattle dehorned by amputation with and without local anaesthesia was not expected. Moreover, meta-regression analyses suggested significant increase in cortisol level in dehorned animals with pain mitigation. One probable explanation is that the injection *per se* before intervention may also influence the interpretation, not primarily due to the punctures itself but presumably due to the pressure caused by the injected volumes (Graf and Senn 1999). Second, even though Schwartzkopf-Genswein *et al.* (2005) and

Graf and Senn (1999) indicated that the handling and restraint associated with dehorning itself did not evoke additional rise in hormone concentration, the increase can happened in animals unaccustomed to handling (Stafford and Mellor 2011; Sinclair 2012). Third, differences existed in the method of achieving the anaesthesia. Most studies block only the perineural space surrounding the cornual nerve (Morisse *et al.* 1995; McMeekan *et al.* 1998; Mellor *et al.* 2002), whereas others attempted to completely desensitize other local nerve blocks, such as ring blocks or caudal horn blocks (Graf and Senn 1999; Faulkner and Weary 1997; Doherty *et al.* 2007; Sinclair 2012). Morisse *et al.* (1995) showed that the effectiveness of anaesthesia was obvious in only 60% of animals in the experiment. Finally, the "ceiling effect" on cortisol secretion can suppress further increase of the more invasive treatments (Mellor *et al.* 2000).

When looking at all studies together which analysed cautery dehorning, there was no consistent evidence of an overall effect on the cortisol levels. A summary effect calculation by the medicaments' classes would be invalid here because the data was scarce to obtain conclusion. The transient increase in cortisol concentration that normally following the procedure was reduced by the administration of local anaesthetic (Mellor and Stafford 1997) or multi-modal therapy (Huber *et al.* 2013), suggesting that the pain management can reduce the pain to the level of the handling of the calves. However, when the hot-iron dehorning was performed without pain management, the increase in cortisol response was greater 30 (Sinclair 2012), 60 (Stilwell *et al.* 2012), and 120 min (Schwartzkopf-Genswein *et al.* 2005) post-treatment than in sham-dehorned group, as well as in calves subjected to dehorning with some pain mitigation (Doherty *et al.* 2007; Stilwell *et al.* 2012). As concluded by Graf and Senn (1999), cattle experienced considerable stress and pain by heat cauterization, with a moderate overall cortisol response (Stafford and Mellor 2005).

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

The effect of dehorning on average daily weight gain

Performance parameters may not replicate the pain experienced by cattle during dehorning, i.e. cannot reflect what is happening to the animal now, but what was happening between observations (Weary *et al.* 2006). If economic gains could balance the cost, pain management at the time of dehorning might be adopted more readily by producers (Newton and O'Connor 2013; Stock *et al.* 2013). However, average daily weight gain as a painful biomarker was likely never intended for this purpose, as showed by our SR-MA.

In agreement to our results, other researchers observed a lack of effect on ADG up to 8 weeks after intervention when dehorned animals by amputation were compared to non-dehorned cattle (Sinclair 2012; Neely *et al.* 2014). Even though amputation dehorning decreased grazing behaviour and increased restlessness, there was no difference in the appetite score nor in the food intake (Sylvester *et al.* 2004; Sinclair 2012; Neely *et al.* 2014). Sinclair (2012) demonstrated that there is a response to the stress of the treatment day, whereby feeding is suppressed to begin with and replaced by locomotion, with feeding resuming to normal level over time. Then, it is reasonable

to assume that the difference in the behaviour, together with cortisol changes, suggests that dehorning cause significant pain in the first 6 h (Sylvester *et al.* 2004).

Despite similar pattern was observed when dehorned animals received anaesthesia, with no differences in the daily feed intake (Newton and O'Connor 2013), local anaesthetic eliminated the behavioural differences, including rumination, during the period of anaesthesia (2 h) (Sylvester *et al.* 2004). On the other hand, the use of anti-inflammatory can impact the performance and the feeding behaviour of calves after cautery dehorning (Faulkner and Weary 2000) and amputation dehorning (Sinclair 2012). Some of the differences in the feeding behaviour, not in physiological ADG indicator *per se*, may be not an effect of medicament itself, but may be consequence beyond the drug's effect.

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

The average effect increased by 34% and decreased by 51%, but remained positive, after the removal two studies published by Sinclair (2012). Those studies had a relatively small sample size per group (n = 9 to 13 cattle), and the precision of estimate was high, which may influence on the average effect. Moreover, a relevant point is the observation period for this outcome (13 and 56 days), since long-term impact of dehorning in ADG is the important question (Newton and O'Connor 2013).

The effect of dehorning on vocalization

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

Increases in vocalization have previously been associated with greater pain during dehorning (Schwartzkopf-Genswein *et al.* 2005) and is in agreement with our results. Neely *et al.* (2014) observed that cattle mechanical dehorning had a greater vocalization scores, and the most extended vocalization, during process than sham dehorned. Although local anaesthetic reduced the vocalization at dehorning, topical anaesthetic was not effective (Sinclair 2012). Moreover, those animals that received local anaesthetic and anti-inflammatory vocalized 7-8 times less during intervention than dehorned without pain alleviation (Sinclair 2012). Traditionally amputation wounds were cauterised to reduce haemorrhage (Stafford and Mellor 2011); however, during the intervention the animals which received topical anaesthetic and had their horn bud cauterized showed significantly more counts of vocalization, and evidences of greater inflammation, tissue damage and slower wound healing rates (Sinclair 2012). A marked increase in other behaviours, such as forcing ahead, rearing and struggling, is strong sign evidence of avoidance and escape and apparently indicative of pain and stress after dehorning, regardless of the instrument used (Graf and Senn 1999; Sinclair 2012).

In spite of Neely *et al.* (2014) observed significant differences in the vocalization score between two different amputation dehorning, in our results there was no effect of

dehorning amputation's techniques on vocalizations' count in cattle. Also, Sinclair (2012) showed no differences between knife and scoop dehorner; however, both vocalized more than animals dehorned with hot-iron. Moreover, there were not statically different for this behaviour if local anaesthetic (Doherty *et al.* 2007) or anti-inflammatory (Faulkner and Weary 2000) was used before hot-iron dehorner.

Even though all studies showed an influence in pooled estimate and on the between studies heterogeneity, speculations about reasons for differences in vocalization did not show any significant effect. Nevertheless, these analyses would have had limited power given the small number of trials available (Borenstein *et al.* 2009). Furthermore, in the way as vocalization was measured, the potential for detection bias was high. This suggests that larger, well-reported field studies are needed to prove that this behaviour is a good pain indicator.

Our study has limitations. First, the approach to reporting outcomes often limited our ability to summarize the data, as there was incomplete reporting of summary measures; then, an attempt was made by contacting scientist in the field (Egger *et al.* 2001). Second, we had to exclude 10 full-text articles about dehorning or disbudding that passed abstract screening as we were unable to translate them. These included articles in German, Norwegian, and Japanese, which might have introduced language bias, because negative findings are published in local journals, i.e. non-English-language reports (Egger *et al.* 2001). Finally, in the absence of a robust and specific measures associated with pain, the choice of indicators about welfare and its relationship with dehorning may be difficult.

In conclusion, this is the first SR-MA that summarized the available literature about effects of dehorning on welfare in beef cattle. We demonstrated that dehorning reduces the welfare of cattle by the increase in cortisol concentration and in the number of vocalizations. Local anaesthesia was effective in reduce the acute stress. The challenges are conduct researches about other effective strategies to alleviate the

stress and pain experienced by dehorned cattle and validates better physiological biomarker of pain.

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CAPÍTULO IV

“It is only with the heart that one can see rightly.
What is essential is invisible to the eye”
(Antoine Saint-Exupéry)

1. CONSIDERAÇÕES FINAIS

O presente estudo pode ser considerado inovador. É a primeira abordagem, com o uso da revisão sistemática-meta-análise, que buscou avaliar efeitos no bem-estar de bovinos de corte decorrentes da castração, da descorna e do amochamento. Essa metodologia tem demonstrado vantagens devido às limitações no tamanho de estudos individuais, já que permite uma análise conjunta de diferentes populações e avaliação de novas hipóteses, mostrando-se de grande valia em relação a qualquer outro estudo isolado. Contudo, vale ressaltar que, nenhum estudo, seja ele meta-analítico ou não, irá permitir uma compreensão definitiva da hipótese de pesquisa.

Diante disso, deixar claro as limitações é imprescindível. A maneira como os resultados foram reportados, além da falta de detalhamento da metodologia e dos resultados, podem ter comprometido a análise. Entretanto, a fim de obter resultados mais fidedignos, esforços foram feitos ao buscar estudos em anais de congressos e ao contatar pesquisadores da área. Devido a limitação em traduzir artigos em determinados idiomas, 16 artigos completos que passaram na triagem inicial dos resumos tiveram que ser excluídos (publicações em alemão, norueguês, búlgaro e japonês). Com isso, pode haver ocorrido a introdução de viés de linguagem, uma vez que achados negativos ou tendem a ser publicados em periódicos locais, em línguas que não a inglesa, ou não são publicados. Por fim, na ausência de indicadores robustos e específicos associados a dor, a escolha de parâmetros sobre o bem-estar e sua relação com a castração e a descorna pode ter sido comprometida.

A avaliação de estratégias para mitigar a dor é uma área em crescimento na literatura científica do século XXI, consequente da maior preocupação pública sobre o tema, além da constante busca por soluções práticas. Entretanto, partindo de um universo amplo de estudos, um pequeno número de publicações foi considerado válido para a análise quantitativa. Dessas, a maioria foi conduzida em países da Oceania e da América do Norte, referências na formulação de códigos para o bem-estar dos animais de produção.

Ademais, o detalhamento da metodologia ainda é falho, mesmo com a existência de guias instrutivos. A falta de discrição do método utilizado para a

alocação dos tratamentos, do uso ou não de cegamento e do protocolo de intervenção foram corriqueiramente observados. Diante disso, é essencial que os autores de estudos controlados estejam mais atentos em prover detalhes completos e precisos dos métodos utilizados.

Partindo, então, para a análise quantitativa dos dados, os principais resultados obtidos foram:

1. Práticas de manejos comuns em fazendas, tanto a castração como a descorna em bovinos de corte com até um ano de idade provocam alteração na fisiologia, no comportamento e no desempenho dos animais;
2. Os níveis de cortisol, 30 e 120 min. após a castração, não foram diferentes entre os grupos castrado e controle;
3. Tanto a castração cirúrgica como a não cirúrgica influenciaram o ganho médio diário de peso dos animais;
4. Uma promessa para a melhoria do bem-estar dos animais, a terapia multi-modal, não foi capaz de reduzir a resposta fisiológica dos bovinos 30 min. após a castração;
5. A literatura identificada e os resultados obtidos não permitiram elucidar qual método e que tipo de estratégia medicamentosa é capaz de minimizar a resposta fisiológica, comportamental e produtiva durante e após a castração;
6. Durante a descorna mecânica, bovinos submetidos ao procedimento sem o uso de medicamento tenderam a vocalizar mais que o grupo controle. Após 30 e 120 min., um aumento substancial dos níveis de cortisol foi observado;
7. Diferentes procedimentos de descorna por amputação promoveram resposta comportamental idêntica nos animais;
8. A descorna manual não comprometeu o desempenho dos animais;
9. Em consonância com achados presentes na literatura, o uso de anestésico local reduziu a elevação imediata do cortisol após a descorna por amputação, típica nesse procedimento, como alternativa na mitigação da dor aguda.

Um dos grandes desafios para um futuro próximo, portanto, é a validação de indicadores de bem-estar dos animais, e de dor, já que são confundidos, com frequência, com a resposta geral ao estresse. A determinação do bem-estar por profissionais baseia-se, frequentemente, na avaliação de parâmetros visuais ou de desempenho. O entendimento de alterações comportamentais associados à dor é de grande valia, porém devem ser interpretados com precaução. Mais estudos são necessários para identificar possíveis comportamentos associados a uma determinada intervenção, considerando natureza do estímulo nocivo, espécie animal, estágio de desenvolvimento e predisposição a dor. Biomarcadores de dor são, geralmente, utilizados em pesquisas científicas e mensurados em amostras biológicas com o uso de protocolos invasivos. A identificação de protocolos minimamente invasivos irá aumentar a nossa capacidade em conduzir estudos com bem-estar animal, além de permitir a obtenção de resultados mais confiáveis.

A dor mais fácil de ser tratada é aquela que nós provocamos. Se esses procedimentos são realizados com frequência, e há um tempo considerável, por que ainda pecamos em minimizar o sofrimento? Como profissionais, temos a obrigação de evitar o início da dor, o estímulo nocivo e sua percepção, além da cascata dor – estresse – desconforto. Por isso, outro desafio para a ciência é a busca por alternativas, farmacológicas ou não, que sejam viáveis, práticas e de fácil uso em rebanho comerciais de bovinos de corte. Se farmacológicas, ainda existem as considerações que regulamentam o uso de compostos analgésicos em animais.

Por isso, vejo como essencial a realização de pesquisas sobre “dor – indicadores - mitigação - bem-estar” para que respostas mais consistente sejam obtidas. Ademais, uma comunicação efetiva entre os diversos grupos envolvidos - produtores, entidade governamentais, cientistas, profissionais, grupos de defesa dos animais, indústrias farmacêuticas – é fundamental para que se obtenha sucesso nessa longa e árdua jornada.

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3. APÊNDICES

APÊNDICE 1: Protocolo utilizado na triagem dos resumos

Relevance Tool I for Screening Abstracts

"A systematic review of the operational and painful procedures that influence the welfare of beef cattle"

Relevance criteria

1. **Does this abstract investigate primary research[1]?**

☐ Yes (include)
☐ Literature Review[2] (exclude)
☐ Other[3] (exclude)
☐ Cannot tell, but likely yes (neutral)

[1] Primary research represents a study where the author(s) collected and analysed their own data.
[2] Literature reviews is a body of text that aims to review the critical points of current published knowledge on a particular topic by accredited scholars or researchers
[3] Other includes: commentaries, books chapters, essay papers, simulation model, conference proceedings on general topics, etc, are excluded

2. **Does this abstract investigate animal welfare in beef cattle[4]?**

☐ Yes (include)
☐ No (exclude)
☐ Cannot tell, but likely yes (neutral)

[4] Are the young of domestic cattle, until twelve months (one year), to produce beef.

3. **Does this abstract investigate one of the following operational procedures in beef cattle (check all that apply)**

☐ Castration[5]
☐ Dehorning[6]
☐ Disbudding[7]
☐ Other (please specify)
☐ Cannot, tell but likely yes
☐ None of the above (exclude)

[5] Is any action - surgical, chemical, hormonal – to remove or destroy the testicles.
[6] Means the amputation of horns at any stage after their growth the progressed beyond the early budding stage.
[7] Refers to prevention of horn growth before it has become advanced.

4. Does this abstract investigate one of the following indicators in beef cattle (check all that apply)

- ☐ Vocalization (neutral)[8]
- ☐ Cortisol (neutral)[9]
- ☐ Weight gain (neutral)
- ☐ Other (please specify)
- ☐ Cannot tell, but likely yes
- ☐ None of above (exclude)

☐ ☐ ☐

[8] It is a behavior measure, which elicit help or stop other animals.

[9] It is a biological response to stress. Changes in plasma cortisol concentration appear to be particularly useful as an index of acute distress.

The reviewer decision is Q1 & Q2 = Yes, Q3 = Castration and/or dehorning, and/or disbudding, Q4= Vocalization and/or cortisol and/or weight gain pass to next level. For all "cannot tell" articles full paper should be obtain

APÊNDICE 2: Formulário utilizado na extração dos dados dos artigos completos

Quality Assessment / Data Extraction (QA/DE) Form for Primary Research Studies Investigating operational and painful procedures that influence the welfare of beef calves

Relevance confirmation based on a full article (questions in this section apply to all studies or study designs)		
Question	**Response options**	**Definitions and /or explanations**
1. What is the language of this paper or publication?	1. English (include) 2. Spanish (include) 3. Portuguese (include) 4. Italian (include) 5. Other (exclude) 6. Can't tell	Please specify "can't tell" if you cannot recognize the language.
2. Was an appropriate control group used?	0. No (exclude) 1. Yes, concurrent control (include) 2. Yes, historical (include) 3. Before and After (include)	**No:** no control group is used, or controls are from a different sampling frame. **Yes, concurrent**: controls are drawn from the same sampling frame (same herd or group) and are measured in the same time frame as treatment group. **Yes, historical:** controls are drawn from same sampling frame (same herd or group), however, they are measured in an earlier time frame than the treatment group, and are not the same samples as treatment group. **Before and after trials**: the study uses the same animal as its own control and take samples before and after the intervention.
3. Is sufficient raw or unadjusted data provided for weight gain and/or cortisol and/or vocalization? (primary data)	0. No, please specify (exclude) 1. Yes, please specify (include) Drop down menu	**0 = No, please specify** Results provided included a. No report of raw results. b. Median. c. Only p-value. d. Only denominator. e. Only nominator. f. Other, please specify. **1 = Yes, please specify** a. Raw results (mean for each group, mean difference, percentage). b. Denominator and nominator.
4. Is measure of dispersion for the raw or unadjusted mean data provided for weight gain and/or cortisol and/or vocalization?	0. No, please specify (exclude) 1. Yes, please specify (include)	**0 = No, please specify** Results provided included a. No report of measure of dispersion and data not available for estimation.

		1 = Yes, please specify a. Measure of dispersion (SD, SE, CI). b. p-value and sample sizes. c. Graphical format from which resources could be extract (will be contact the author(s) to obtain the data).
5. Is sufficient raw or adjusted data provided for weight gain and/or cortisol and/or vocalization?	0. No, please specify (exclude) 1. Yes, please specify (include) 2. Not applicable	**0 = No, please specify** Results for the multivariable model for one or all the outcomes measures (weight gain and/or cortisol and/or vocalization): a. Were not provided. b. Were not presented in a extractable format (graph from which results cannot be extracted). c. Included measures of effect but no measures of variability were presented. **1. Yes** a. Results of the multivariable model are presented for at least one of the outcome study. b. Included parameters estimate (mean, mean difference) and Measure of variability (SE, SD, CI) or p-value are provided after adjusting for other variables, confounders and/or clustering and Sample size (n) reported. **2. Not applicable** No multivariable was used.
6. If the paper was excluded, why?	……	Write the motive(s) which the paper was excluded. Specify: MA or RS qualitative?
Note: If the language of the paper is "Other" or the study contains no control group or there are not numbers to extract (because they were not provided), please do not proceed with the rest of the evaluation and submit your answers.		
GENERAL STUDY INFORMATION		
Ref ID	……	Captured in SRS
Journal name	……	
Author(s)(s) name	……	
Publication year	……	
7. What is the study design as identified by the reviewer?	0. Quasi-experiment (QE) 1. Controlled trial (CT) 2. Cohort study 3. Other (please specify)	**QE**: before and after trials, including indicators evaluation at various points (before and after one or more stages). **CT**: a planned experiment. May or may not be randomized. Lab. trial= executed under highly controlled conditions. Field trial= executed

		under less controlled, more "real" conditions. **Cohort study**: a group of animals exposed to a hypothesized risk factor (exposure), and a group not exposed to the factor are selected and observed during the study period to record the indicator(s) (weight gain and/or cortisol and/or vocalization) in each group. **Other**: hybrid, for example cross-over which can be used to evaluate an intervention or other.
Note: If there are two design, STOP reviewing and contact Maria Eugênia A. Canozzi. The form will need to be cloned and the two different studies will need to be extracted separately.		
8. Publication type	1. Peer reviewed 2. Conference proceedings 3. Thesis 4. Government or research stations report 5. Other (please specify) …….	
9. Country/region/province/state were the study was carried out	1. …….	If the study/sampling location is not reported in the paper, please use the primary author(s) affiliation. If author(s) affiliation indicates more than one country/region/province, please record all.
10. In what year(s) was the data collected	1. Please enter 2. Not reported	**1.** Please do not use the publication date to answer this question. Please record answer as stated in the text of the paper. If not reported don't record. **2. Not reported**: the author(s) did not report the year of data collection.
11. Institution that funded the study	1. …….. 2. Not reported	Please do not use author(s) affiliation to answer this question.
12. How were <u>operations (mainly for commercial cattle farms) selected</u> to participate in this study?	0. Not reported 1. Random 2. Reported random 3. Systematic 4. Convenience or purposively 5. Not applicable	**Random**: computer or random number table, a priori, stratified random sample, cluster random sample (complete list of the target population is required). **Reported random**: author(s) report random, but randomization is not explained. **Systematic**: taken n samples at interval of x (complete list of the target population is not required). **Convenience**: participants were identified by personal contacts or responded to a survey. **Purposive**: participants identified based on the elements possessing one or more attributes. **Not applicable**: operations were not selected to participate in this

		trial, this is mainly a Research facility/University station trial.
13. Within the operations, was herd, pen or paddock selection described and justified?	0. Not reported 1. Random 2. Reported random 3. Systematic 4. Convenience or purposively 5. Not applicable	**Random**: computer or random number table, a priori, stratified random sample, cluster random sample (complete list of the target population is required). **Reported random**: author(s) report random, but randomization is not explained. **Systematic**: taken n samples at interval of x (complete list of the target population is not required). **Convenience**: participants were identified by personal contacts or responded to a survey. **Purposive**: participants identified based on the elements possessing one or more attributes. **Not applicable**: operations, flocks or pen were not selected for the study.
14. Within the operations, herds, pens or paddocks, were calves selection described and justified?	0. Not reported 1. Random 2. Reported random 3. Systematic 4. Convenience or purposively 5. Not applicable	**Random**: computer or random number table, a priori, stratified random sample, cluster random sample (complete list of the target population is required). **Reported random**: author(s) report random, but randomization is not explained. **Systematic**: taken n samples at interval of x (complete list of the target population is not required). **Convenience**: participants were identified by personal contacts or responded to a survey. **Purposive**: participants identified based on the elements possessing one or more attributes, such as age and/or weight and/or breed. **Not applicable**: calves were not selected for the study.
15. Was the sample size justified at the operation (farm) level?	0. No 1. Yes 2. Not applicable	**Yes**: use sample-size formulas, based on a desired power or precision and estimate of expected variability to detect differences, or the author(s) justified the sample population is the census population/maximum accessible or costs and welfare protocols? **No**: no details in the text regarding how the number of farms/ herds /pens were determined or the author(s) describes informal guesses of sample size. **Not applicable:** farms/herds/pens were not selected to participate in the study.

16. Was the sample size justified at the individual level?	0. No 1. Yes 2. Not applicable	**No**: no details in the text regarding how sample size was determined or the author(s) describes informal guesses of sample size. **Yes**: use sample-size formulas, based on a desired power or precision and estimate of expected variability to detect differences, or the author(s) justified the sample population is the census population/maximum accessible or costs and welfare protocols? **Not applicable:** calves were not selected to participate in the study.
17. List the inclusion/exclusion criteria	1. Please enter 2. Not reported	1. Please specify inclusion/exclusion criteria as reported by author(s). 2. No inclusion/exclusion criteria were reported.
STUDY POPULATION(S)		
18. What was the setting for the study?	0. Not reported 1. Commercial cattle farm 2. Research farm 3. Research facility	**Commercial cattle farm**: the study was conducted on a farm that is raising cattle for commercial purpose. **Research farm**: the study is conducted on a farm belonging to a Research Institution/University/College. **Research facility**: the study is conducted at an Institution which has animal facilities for experimentation.
19. What is the gender of the calves in the study?	1. Female 2. Male 3. Not reported	**Female calves:** are young female cattle until 12 months of age. **Male calves:** are young male cattle until 12 months of age.
20. What is the breed of cattle?	1. Please enter 2. Not reported	Please specify as reported by the author(s).
21. What is the age (in days) of the calves in the experiment?	1. Please enter 2. Not reported	Please specify as reported by the author(s).
22. Please indicate the total number of each included in the study.	1. Herd ……. 2. Individual cattle ……. 3. Other (please specify) ……. 4. Not reported	Please provided information as reported. **Herd:** are group of cattle raised together on a farm. **Individual animals:** specify number of calves included in the study.
INTERVENTION(S)		
23. What type of intervention(s) is the author(s) studying?	1. Castration 2. Dehorning 3. Disbudding 4. Other	**Castration:** is any action - surgical, chemical, with small rubber rings, with clamps (Burdizzo) – to remove or destroy the testicles. **Dehorning:** means the amputation of horns at any stage after their growth the progressed beyond the early budding stage.

		Disbudding: refers to prevention of horn growth before it has become advanced. **Other**: if the study reports another intervention besides the listed here please specify and extract the data. Please specify in the textbox type of intervention.
24. Who performed the procedure?	0. Not reported 1. Farm staff 2. Veterinarian 3. Other (please specify) …….	Please specify as reported by the author(s).
25. Which type of medicament was applied for performing the intervention?	1. Parenteral analgesic ……. 2. Sedative ……. 3. Local anaesthetic ……. 4. Regional anaesthetic ……. 5. Anti-inflammatory ……. 6. Not applicable	Please specify the parenteral analgesic, sedative, anaesthetic or anti-inflamatory was used as reported by the author(s)(s). **Not applicable:** medicament was not used in the study.
26. How was the intervention assigned to the experimental unit?	0. Convenience 1. Random 2. Reported random 3. Systematic	**Convenience**: assignment is not reported in the paper. **Random**: computer or random number table, *a priori.* **Reported random**: author(s) report random, but randomization is not explained. **Systematic**: taken n samples at interval of x.
27. Were the intervention protocols described in sufficient detail to allow reproduction of the experiment?	0. Yes 1. Reference paper 2. No	**Yes:** methods are thoroughly described and allow for replication. **Need to find a reference paper:** methods are referenced in another paper. **No**: necessary information is missing.
28. What was the first time before intervention to measurement each of the following outcomes?	0. Cortisol ……. 1. Vocalization ……. 2. Weight gain ……. 3. Other	**Other**: if the study reports another outcome besides the listed here please specify and extract the data.
29. Describe the treatment groups	1. Castration ……. 2. Dehorning ……. 3. Disbudding ……. 4. Other	Describe the comparative groups for each study intervention as describe by the author(s) (e.g. G1: control, G2: surgical, G3: Burdizzo).
30. Did the author(s) report that blinding was used to treatment groups allocation?	0. No 1. Yes	**Yes:** the author(s) reported that blinding was used. **No:** no blinding was reported.
OUTCOME MEASUREMENTS		
31. Which was the outcome evaluation?	1. Vocalization 2. Cortisol 3. Weight gain 4. Other …….	**Other**: if the study reports another intervention besides the listed here please specify.
32. Which type of sample was tested in this study?	1. Blood samples 2. Visual / Direct	

	observation 3. Video camera 4. Fecal samples 5. Urine samples 6. Saliva samples 7. Other (please specify)	
33. Was the outcome evaluated before the intervation?	1. Cortisol (yes, no, no reported) ……. 2. Vocalization (yes, no, no reported) ……. 3. Weight gain (yes, no, no reported) ……. 4. Other	**Yes:** author(s) reported that the outcome was evaluated before the intervation. **No:** author(s) reported that the outcome was not measure before the intervation. **No reported:** there is no infromation about the outcome was measure before the intervation.
34. What method was used to evaluate the following outcomes?	1. Cortisol ……. 2. Vocalization ……. 3. Weight gain ……. 4. Other	**Vocalization:** *Ad libitum / All occurrences sampling:* records as much information as possible. *Focal animal sampling:* all occurrence of specified action of one individual are recorded during a predetermined sample period (e.g., one hour). *All occurrence sample:* the observer focuses on a particular behaviour rather than in a particular individual. *One-zero sampling:* the researcher records whether specific behaviours did (1) or did not (0) occur during a given time interval. *Instantaneous or scan sampling:* an animal's activities are recorded at pre-selected moments (e.g., every 30 seconds). **Cortisol:** radioimmunoassay, immunoassay technique, enzimeimmunoassay, modified enzimeimmunoassay. **Weight gain:** manual weighting (on manual or electronic balance), automatic weighting (GrowSafe™ Systems), using body measurements, by image analysis. Please, specify the evaluation method for each outcome as reported by the author(s).
35. Was there cortisol cut-off level that was used as stress indicator?	0. No 1. Yes ……. 2. Not reported	**No: :** the author(s) report that there is a cortisol cut-off level, but did not report the value. **Yes:** please enter the report value. **Not reported:** the author(s) did not report any cortisol cut-off level could will be used as stress indicator.
36. Did the author(s) report that blinding was used to evaluate the	0. No 1. Yes	**No:** no blinding was reported. **Yes:** the author(s) reported that

outcome?	2. Not applicable	blinding was used. **Not applicable:** the author(s) reported why the blinding was not used.
37. Based on the study design, was clustering accounted for appropriately in the analysis?	0. No 1. Yes 2. Not applicable	**No:** the data is clustered (group hierarchy, repeated measures or multiple replicates exist and data is pooled/collapsed), and clustering was not controlled for. **Yes:** clustering may be as a result of *group hierarchy, repeated measures, or multiple replicates*. Clustering was present and was accounted for (e.g. fixed or random effect in the model, Generalized Estimating Equations - GEE, Generalized Linear Mixed Models - GLMMs, Markov chain Monte Carlo - MCMC, robust variance estimation, linear mixed model, over dispersion factor, ANOVA, Bayesian mixed models). *Group hierarchy (organizational structure):* data is clustered by group, for example, farm or pen. *Repeated measures:* several measurements of an outcome are taken on the same unit of observation over a period of time. *Multiple replicates:* the experiment was conducted more than once. **Not applicable:** there was no clustering present or group hierarchy, repeated measures or multiple replicates exist, but data is not pooled/collapsed.
38. Were identified confounders controlled for or tested?	0. No 1. Yes, analysis 2. Yes, inclusion/exclusion 3. Yes, matching 4. Not applicable	**Yes:** confounders were identified by the author(s) and tested in the analysis (for example Mantel-Haenszel test - M-H; regression analysis) for their impact on the outcome, or taken care of in the inclusion/exclusion criteria or by matching. **No:** no adjustment was made for confounders/effect modifiers, etc, that were identified by the author(s). **Not applicable:** there were no confounders identified by the author(s) in this study, or the study used randomization to control for confounders.
39. Was the statistical analysis described adequately so it can be reproduced?	0. No 1. Yes 2. Reference paper 3. Statistical analysis not done	**Yes:** the methods were reported in sufficient detail to understand the statistical approach and reasoning. **Need to find referenced paper**: methods are referenced in another

				paper. **No:** methods and adjustments are not clear or some details are missing. **Statistical analysis not done:** no statistical analysis was done.
RESULTS				
40. What <u>secondary outcomes</u>, if any, were measured in this study?	……			Please specify any outcome other than weight gain and/or cortisol and/or vocalization . Please <u>do not provide any data on these outcomes</u>.
41. Trial/Experiment ID				Please specify each trial conducted (e.g. T1: castration, T2: dehorning,…Tn)
42. Outcome type/unit of measure/time				Please provide information as reported. For example, cortisol mean or etc., unit of measure (nmol/L, kg) and the time when was measured (e.g. 0 min, 10 min, 20 min, etc.).
Raw or unadjusted data				**In this section we use the raw data without process**
43. Groups/Trial				Please specify the treatment groups per trial (e.g. G0= control; G1= Burdizzo; G2= Burdizzo + analgesic, etc).
44. Total samples per outcome				The total number of samples analysed per outcome in the study. Please identify with relation to comparison groups (e.g., cortisol: G0= 10, G1= 8, G2= 10… Gn).
45. Cortisol or vocalization or weight gain or other <u>mean</u>				Mean and unit (or another measure). Please specify which one and identify with relation to comparison groups (e.g., cortisol: G0= 40 nmol/L, G1= 50 nmol/L, G2= 45 nmol/L… Gn). Please provide information as provided by author(s).
46. Cortisol or vocalization or weight gain or other <u>SE or SD</u>				Standard error and/or standard deviation (please indicate which). Please identify with relation to comparison groups (e.g., cortisol: SE: G0= 2, G1= 5, G2= 6.7… Gn).
47. Cortisol or vocalization or weight gain or other <u>CI</u>				95% confidence interval. Please identify with relation to comparison groups (G0, G1, G2... Gn).
48. <u>p-value</u>				Please specify the p-value for the comparison between groups of reported by the author(s) (e.g., control vs. G1= $p<0.05$, favor G1).
Statistical analysis				
49. Type of analysis				The model or statistical test conducted to give the measure of precision and p-value. For

				example, t-test, chi-square, ANOVA.
50. Model adjusted by, if applicable				List the random effects and fixed effects included in the model for these results.
51. Outcome type				Please specify the outcome. For example, binary (Odds Ratios - OR, Risk Ratios - RR), or continuous (mean difference, LS means). This may also include log OR, or a coefficient in the log or other transformed scale.
52. Estimate of effect for each of the reported outcomes				Indicate the result of the test or model. If applicable add interpretation to the estimate (e.g. $p<0.05$ favours treatment in relation to control).
53. Measure of variability of effect estimate				Indicate the result of the test or model (other results and interpretation).
54. p-value and interpretation				The level of significance achieved by the test interpretation (e.g. $p<0.05$ Tx lowered cortisol, etc).
55. Additional comments *(If the reviewer feels there is something that was not captured in the tool but should be acknowledged in QA or DE)*	T-box			

APÊNDICE 3: Base de dados utilizada na meta-análise sobre os efeitos da castração em indicadores de BEA

Ganho médio diário de peso

study	author	surg	med_type	group_cont	surg_cont	group_tx	n_ cont	mean_ cont	sd_ cont	n_ tx	mean_ tx	sd_ tx
1	Baker et al., 2000	non-surgical	NA	intact	not castrated	ring	20	780	200	18	760	210
1	Baker et al., 2000	surgical	NA	intact	not castrated	knife	20	780	200	22	770	230
1	Baker et al., 2000	combined	NA	ring	non-surgical	knife	18	760	210	22	770	230
4	Coetzee et al., 2012	surgical	NA	intact	not castrated	knife & emasculator	53	1540	1310	73	1160	1580
4	Coetzee et al., 2012	surgical	anti-inflammatory	intact	not castrated	knife & emasculator + meloxicam	53	1540	1310	67	1220	1490
4	Coetzee et al., 2012	surgical	NA	intact + meloxicam	not castrated	knife & emasculator	57	1540	1310	73	1160	1580
4	Coetzee et al., 2012	surgical	anti-inflammatory	intact + meloxicam	not castrated	knife & emasculator + meloxicam	57	1480	1350	67	1220	1490
4	Coetzee et al., 2012	surgical	anti-inflammatory	knife & emasculator	surgical	knife & emasculator + meloxicam	73	1160	1580	67	1220	1490
10	Faulkner et al., 1992	surgical	NA	intact	not castrated	knife	134	420	490	134	260	490
12	Fisher et al., 2001	surgical	anaesthetic	intact + lido	not castrated	knife & emasculator + lido	20	280	140	10	370	140
12	Fisher et al., 2001	non-surgical	anaesthetic	intact + lido	not castrated	ring + lido	20	280	190	10	480	190
12	Fisher et al., 2001	combined	anaesthetic	ring + lido	non-surgical	knife & emasculator + lido	10	480	140	10	370	140
13	González et al., 2010	non-surgical	NA	sham	not castrated	ring	23	970	380	22	680	380
13	González et al., 2010	non-surgical	NA	sham + xyl & flunixin	not castrated	ring	20	890	380	22	680	380
13	González et al., 2010	non-surgical	multi-modal	sham	not castrated	ring + xyl & flunixin	23	970	380	24	680	380
13	González et al., 2010	non-surgical	multi-modal	sham + xyl & flunixin	not castrated	ring + xyl & flunixin	20	890	380	24	680	380
13	González et al., 2010	non-surgical	multi-modal	ring	non-surgical	ring + xyl & flunixin	22	680	380	24	2650	380
19	Pang et al., 2008	non-surgical	anaesthetic	sham + lido	not castrated	ring + lido	80	980	430	80	580	430
19	Pang et al., 2008	non-surgical	anaesthetic	sham + lido	not castrated	emasculator + lido	80	980	430	83	650	430

19	Pang et al., 2008	non-surgical	anaesthetic	ring + lido	non-surgical	emasculator + lido	80	580	430	83	650	430
21	Warnock et al., 2012	non-surgical	NA	intact	not castrated	ring	15	960	570	15	800	570
21	Warnock et al., 2012	surgical	NA	intact	not castrated	knife & Hend	15	960	570	15	860	570
21	Warnock et al., 2012	surgical	NA	intact	not castrated	knife & emasculator	15	960	570	15	880	570
21	Warnock et al., 2012	combined	NA	ring	non-surgical	knife & Hend	15	800	570	15	860	570
21	Warnock et al., 2012	combined	NA	ring	non-surgical	knife & emasculator	15	800	570	15	880	570
21	Warnock et al., 2012	surgical	NA	knife & emasculator	surgical	knife & Hend	15	860	570	15	880	570
22	Reppening et al., 2013	combined	NA	ring	non-surgical	knife & Hend	10	910	670	10	1130	670
23	Pieler et al., 2013	surgical	multi-modal	intact + xyl	not castrated	knife + xyl & procaine	10	360	232	10	540	232
23	Pieler et al., 2013	non-surgical	multi-modal	intact +xyl	not castrated	emasculator + xyl & procaine	10	360	116	10	450	116
23	Pieler et al., 2013	surgical	multi-modal	intact + xyl	not castrated	knife & emasculator + xyl & procaine	10	360	116	10	270	116
23	Pieler et al., 2013	combined	multi-modal	emasculator + xyl & procaine	non-surgical	knife + xyl & procaine	10	450	116	10	540	116
23	Pieler et al., 2013	surgical	multi-modal	knife + xyl & procaine	surgical	knife & emasculator + xyl & procaine	10	540	348	10	270	348
23	Pieler et al., 2013	combined	multi-modal	emasculator + xyl & procaine	non-surgical	knife & emasculator + xyl & procaine	10	450	232	10	270	232
26	Patherick, 2011	combined	NA	ring	non-surgical	knife	16	390	51	16	360	51
33	del Campo et al., 2014	non-surgical	NA	sham	not castrated	ring	9	860	110	9	970	110
33	del Campo et al., 2014	surgical	NA	sham	not castrated	knife	9	860	61	9	810	61
33	del Campo et al., 2014	surgical	anaesthetic	sham	not castrated	knife + lido	9	960	340	9	2000	690
33	del Campo et al., 2014	combined	NA	ring	non-surgical	knife	9	970	160	9	810	160
33	del Campo et al., 2014	combined	anaesthetic	ring	non-surgical	knife + lido	9	970	97	9	890	97
33	del Campo et al., 2014	surgical	anaesthetic	knife	surgical	knife + lido	9	810	97	9	890	97
34	del Campo et al., 2014	surgical	NA	sham	not castrated	knife	9	610	49	9	570	49
34	del Campo et al., 2014	surgical	anti-inflammatory	sham	not castrated	knife + dexa	9	610	40	9	590	40

34	del Campo et al., 2014	non-surgical	NA	sham	not castrated	ring	9	610	70	9	540	70
34	del Campo et al., 2014	non-surgical	NA	sham	not castrated	emasculator	9	610	330	9	630	330
34	del Campo et al., 2014	surgical	multi-modal	sham	not castrated	knife + lido & dypirone	9	610	31	9	600	31
34	del Campo et al., 2014	surgical	anti-inflammatory	knife	surgical	knife + dexa	9	570	61	9	590	61
34	del Campo et al., 2014	combined	NA	ring	non-surgical	knife	9	570	59	9	540	59
34	del Campo et al., 2014	combined	NA	emasculator	non-surgical	knife	9	630	73	9	570	73
34	del Campo et al., 2014	surgical	multi-modal	knife	surgical	knife + lido & dypirone	9	570	92	9	600	92
34	del Campo et al., 2014	combined	anti-inflammatory	ring	non-surgical	knife + dexa	9	540	61	9	590	61
34	del Campo et al., 2014	combined	anti-inflammatory	emasculator	non-surgical	knife + dexa	9	630	79	9	590	79
34	del Campo et al., 2014	surgical	multi-modal	knife + dexa	surgical	knife + lido & dypirone	9	590	166	9	600	166
34	del Campo et al., 2014	non-surgical	NA	ring	non-surgical	emasculator	9	540	65	9	630	65
34	del Campo et al., 2014	combined	multi-modal	ring	non-surgical	knife + lido & dypirone	9	540	73	9	600	73
34	del Campo et al., 2014	combined	multi-modal	emasculator	non-surgical	knife + lido & dypirone	9	630	59	9	600	59
35	del Campo et al., 2014	surgical	NA	knife	surgical	knife & emasculator	9	130	24	9	-110	24
35	del Campo et al., 2014	surgical	anaesthetic	knife	surgical	knife + lido	9	130	120	9	-120	120
35	del Campo et al., 2014	surgical	anaesthetic	knife & emasculator	surgical	knife + lido	9	-110	120	9	-120	120
36	del Campo et al., 2014	surgical	NA	sham	not castrated	knife	8	-420	110	8	-370	110
36	del Campo et al., 2014	surgical	anti-inflammatory	sham	not castrated	knife + dexa	8	-420	140	8	-360	140
36	del Campo et al., 2014	surgical	anti-inflammatory	sham	not castrated	knife + dypirone	8	-420	140	8	-360	140
36	del Campo et al., 2014	surgical	multi-modal	sham	not castrated	knife + lido & dypirone	8	-420	150	8	-390	150
36	del Campo et al., 2014	surgical	anti-inflammatory	knife	surgical	knife + dexa	8	-370	150	8	-360	150
36	del Campo et al., 2014	surgical	anti-inflammatory	knife	surgical	knife + dypirone	8	-370	150	8	-360	150

36	del Campo et al., 2014	surgical	multi-modal	knife	surgical	knife + lido & dypirone	8	-370	100	8	-390	100
36	del Campo et al., 2014	surgical	anti-inflammatory	knife + dexa	surgical	knife + dypirone	8	-360	0	8	-360	0
36	del Campo et al., 2014	surgical	multi-modal	knife + dexa	surgical	knife + lido & dypirone	8	-360	150	8	-390	150
36	del Campo et al., 2014	surgical	multi-modal	knife + dypirone	surgical	knife + lido & dypirone	8	-360	87	8	-390	87
37	del Campo et al., 2014	surgical	NA	sham	not castrated	knife	10	260	13	10	270	13
37	del Campo et al., 2014	surgical	anti-inflammatory	sham	not castrated	knife + dexa	10	260	150	10	120	150
37	del Campo et al., 2014	non-surgical	NA	sham	not castrated	ring	10	260	90	10	190	90
37	del Campo et al., 2014	non-surgical	NA	sham	not castrated	emasculator	10	260	170	10	100	170
37	del Campo et al., 2014	surgical	anaesthetic	sham	not castrated	knife + lido	10	260	77	10	200	77
37	del Campo et al., 2014	surgical	anti-inflammatory	knife	surgical	knife + dexa	10	270	160	10	120	160
37	del Campo et al., 2014	combined	NA	ring	non-surgical	knife	10	190	100	10	270	100
37	del Campo et al., 2014	combined	NA	emasculator	non-surgical	knife	10	100	180	10	270	180
37	del Campo et al., 2014	surgical	anaesthetic	knife	surgical	knife + lido	10	270	90	10	200	90
37	del Campo et al., 2014	combined	anti-inflammatory	ring	non-surgical	knife + dexa	10	190	70	10	120	70
37	del Campo et al., 2014	combined	anti-inflammatory	emasculator	non-surgical	knife + dexa	10	100	26	10	120	26
37	del Campo et al., 2014	surgical	anaesthetic	knife + dexa	surgical	knife + lido	10	120	80	10	200	80
37	del Campo et al., 2014	non-surgical	NA	ring	non-surgical	emasculator	10	190	96	10	100	96
37	del Campo et al., 2014	combined	anaesthetic	ring	non-surgical	knife + lido	10	190	13	10	200	13
37	del Campo et al., 2014	combined	anaesthetic	emasculator	non-surgical	knife + lido	10	100	110	10	200	110
39	Martí, 2012	non-surgical	NA	intact	not castrated	ring	20	1490	210	20	1040	210
39	Martí, 2012	non-surgical	NA	intact	not castrated	anti GnRH vaccine	20	1490	210	20	1230	210
39	Martí, 2012	non-surgical	NA	ring	non-surgical	anti GnRH vaccine	20	1040	210	20	1230	210

43	Whitlock et al., 2013	non-surgical	NA	intact	not castrated	ring	16	690	120	16	150	110
43	Whitlock et al., 2013	non-surgical	anti-inflammatory	intact	not castrated	ring + meloxicam	16	690	120	16	140	110
43	Whitlock et al., 2013	non-surgical	anti-inflammatory	ring	non-surgical	ring + meloxicam	16	150	110	16	140	110

Concentração de cortisol

study	author	surg	med_type	group_cont	surg_cont	group_tx	time	n_cont	mean_cont	sd_cont	n_tx	mean_tx	sd_tx
6	Coetzee et al., 2010	surgical	NA	sham	not castrated	knife & Hend	30	4	227.7	75.2	6	170.8	57.4
6	Coetzee et al., 2010	surgical	NA	sham	not castrated	knife & Hend	120	4	154.0	32.4	6	108.6	16.4
6	Coetzee et al., 2010	surgical	analgesic	sham	not castrated	knife & Hend + xyl	30	4	227.7	75.2	6	167.6	65.4
6	Coetzee et al., 2010	surgical	analgesic	sham	not castrated	knife & Hend + xyl	120	4	154.0	32.4	6	129.8	16.7
6	Coetzee et al., 2010	surgical	anaesthetic	sham	not castrated	knife & Hend + xyl & ketam	30	4	227.7	75.2	6	132.0	13.7
6	Coetzee et al., 2010	surgical	anaesthetic	sham	not castrated	knife & Hend + xyl & ketam	120	6	154.0	32.4	6	138.0	20.0
6	Coetzee et al., 2010	surgical	analgesic	knife & Hend	surgical	knife & Hend + xyl	30	6	170.8	57.4	6	167.6	65.4
6	Coetzee et al., 2010	surgical	analgesic	knife & Hend	surgical	knife & Hend + xyl	120	6	108.6	16.4	6	129.8	16.7
6	Coetzee et al., 2010	surgical	anaesthetic	knife & Hend	surgical	knife & Hend + xyl & ketam	30	6	170.8	57.4	6	132.0	13.7
6	Coetzee et al., 2010	surgical	anaesthetic	knife & Hend	surgical	knife & Hend + xyl & ketam	120	6	108.6	16.4	6	138.0	20.0
6	Coetzee et al., 2010	surgical	anaesthetic	knife & Hend + xyl	surgical	knife & Hend + xyl & ketam	30	6	167.6	65.4	6	132.0	13.7
6	Coetzee et al., 2010	surgical	anaesthetic	knife & Hend + xyl	surgical	knife & Hend + xyl & ketam	120	6	129.8	16.7	6	138.0	20.0
7	Coetzee et al. 2008	surgical	NA	sham	not castrated	knife & Hend	30	5	92.0	66.1	5	84.1	50.0
7	Coetzee et al. 2008	surgical	NA	sham	not castrated	knife & Hend	120	5	61.5	45.9	5	63.5	42.6
11	Fell et al., 1986	non-surgical	NA	ring	non-surgical	knife	30	10	0.9	0.7	9	3.9	7.2
11	Fell et al., 1986	combined	NA	ring	non-surgical	knife	120	10	1.2	0.8	9	4.8	9.5

13	González et al., 2010	non-surgical	multi-modal	ring	non-surgical	ring + xyl & flunixin	30	8	7.1	5.8	8	3.9	4.6
13	González et al., 2010	non-surgical	multi-modal	ring	non-surgical	ring + xyl & flunixin	120	8	6.8	5.2	8	3.3	3.9
20	Thüer et al., 2007	non-surgical	anaesthetic	ring	non-surgical	ring + lido	30	10	29.0	15.5	15	21.5	14.4
20	Thüer et al., 2007	non-surgical	anaesthetic	ring	non-surgical	ring + lido	120	10	20.0	10.8	15	14.0	8.7
20	Thüer et al., 2007	non-surgical	anaesthetic	emasculator	non-surgical	emasculator + lido	30	10	37.3	30.4	15	27.5	16.6
20	Thüer et al., 2007	non-surgical	anaesthetic	emasculator	non-surgical	emasculator + lido	120	10	11.3	11.1	15	18.5	12.2
23	Pieler et al., 2013	surgical	multi-modal	intact + xyl	not castrated	knife + xyl & procaine	120	10	3.9	2.6	10	2.9	2.0
23	Pieler et al., 2013	non-surgical	multi-modal	intact + xyl	not castrated	emasculator + xyl & procaine	30	10	3.6	1.9	10	4.1	2.2
23	Pieler et al., 2013	surgical	multi-modal	intact + xyl	not castrated	knife & emasculator + xyl & procaine	30	10	3.6	2.8	10	1.9	2.5
23	Pieler et al., 2013	surgical	multi-modal	intact + xyl	not castrated	knife & emasculator + xyl & procaine	120	10	3.9	2.9	10	2.4	2.6
23	Pieler et al., 2013	surgical	multi-modal	knife + xyl & procaine	surgical	knife & emasculator + xyl & procaine	120	10	3.0	1.5	10	2.4	1.8
23	Pieler et al., 2013	combined	multi-modal	emasculator + xyl & procaine	non-surgical	knife & emasculator + xyl & procaine	30	10	4.1	3.6	10	1.9	3.1
26	Patherick, 2011	non-surgical	anti-inflammatory	ring	non-surgical	ring + ketop	30	8	38.0	16.8	8	43.5	21.9
26	Patherick, 2011	non-surgical	anti-inflammatory	ring	non-surgical	ring + ketop	120	8	44.3	21.2	8	45.5	23.5
26	Patherick, 2011	combined	NA	ring	non-surgical	knife	30	8	38.0	14.6	8	39.5	16.2
26	Patherick, 2011	combined	NA	ring	non-surgical	knife	120	8	44.3	23.5	8	34.0	14.9
26	Patherick, 2011	combined	anti-inflammatory	ring	non-surgical	knife + ketop	30	8	38.0	14.5	8	39.0	15.6
26	Patherick, 2011	combined	anti-inflammatory	ring	non-surgical	knife + ketop	120	8	44.3	24.5	8	32.0	15.2
26	Patherick, 2011	combined	anti-inflammatory	ring + ketop	non-surgical	knife	30	8	43.5	21.1	8	39.5	17.2
26	Patherick, 2011	combined	anti-inflammatory	ring + ketop	non-surgical	knife	120	8	46.5	26.6	8	34.0	16.6
26	Patherick, 2011	combined	anti-inflammatory	ring + ketop	non-surgical	knife + ketop	30	8	43.5	21.3	8	37.0	17.0
26	Patherick, 2011	combined	anti-inflammatory	ring + ketop	non-surgical	knife + ketop	120	8	46.5	27.7	8	32.0	17.2
26	Patherick, 2011	surgical	anti-inflammatory	knife	surgical	knife + ketop	30	8	39.5	16.0	8	39.0	15.5

26	Patherick, 2011	surgical	anti-inflammatory	knife	surgical	knife + ketop	120	8	34.0	10.8	8	32.0	8.8
27	Patherick, 2012	surgical	NA	sham	not castrated	knife	30	10	37.9	14.5	10	37.9	21.6
27	Patherick, 2012	surgical	NA	sham	not castrated	knife	120	10	24.0	14.2	10	25.8	14.6
27	Patherick, 2012	non-surgical	NA	sham	not castrated	ring	30	10	37.9	9.9	9	37.1	14.9
27	Patherick, 2012	non-surgical	NA	sham	not castrated	ring	120	10	25.0	11.8	9	27.5	10.6
27	Patherick, 2012	combined	NA	ring	non-surgical	knife	30	9	37.1	14.1	10	37.9	18.1
27	Patherick, 2012	combined	NA	ring	non-surgical	knife	120	9	27.5	4.5	10	25.8	5.9
28	Patherick, 2012	surgical	NA	sham	not castrated	knife	30	10	39.2	11.9	10	36.7	15.8
28	Patherick, 2012	surgical	NA	sham	not castrated	knife	120	10	23.8	15.0	10	27.5	14.8
28	Patherick, 2012	non-surgical	NA	sham	not castrated	ring	30	10	39.2	23.4	8	24.6	20.9
28	Patherick, 2012	non-surgical	NA	sham	not castrated	ring	120	10	23.7	22.1	8	37.1	24.6
28	Patherick, 2012	combined	NA	ring	non-surgical	knife	30	8	24.6	22.1	10	36.7	26.6
28	Patherick, 2012	combined	NA	ring	non-surgical	knife	120	8	37.1	17.0	10	27.5	12.7
33	del Campo et al., 2014	non-surgical	NA	sham	not castrated	ring	120	9	3.5	3.7	9	3.5	1.2
33	del Campo et al., 2014	surgical	NA	sham	not castrated	knife	120	9	3.5	3.7	9	12.0	41.8
33	del Campo et al., 2014	surgical	anaesthetic	sham	not castrated	knife + lido	120	9	3.5	3.7	9	13.4	19.2
33	del Campo et al., 2014	combined	NA	ring	non-surgical	knife	120	9	3.5	1.2	9	12.0	41.8
33	del Campo et al., 2014	combined	anaesthetic	ring	non-surgical	knife + lido	120	9	3.5	1.2	9	13.4	19.2
33	del Campo et al., 2014	surgical	anaesthetic	knife	surgical	knife + lido	120	9	12.0	41.8	9	13.4	19.2
35	del Campo et al., 2014	surgical	NA	knife	surgical	knife & emasculator	120	9	3.7	2.5	9	4.4	1.9
35	del Campo et al., 2014	surgical	anaesthetic	knife	surgical	knife + lido	120	9	3.7	2.5	9	4.8	2.7
35	del Campo et al., 2014	surgical	anaesthetic	knife & emasculator	surgical	knife + lido	120	9	4.4	1.9	9	4.8	2.7
39	Martí, 2012	non-surgical	NA	intact	not castrated	ring	30	20	2.8	0.4	20	3.9	1.1
39	Martí, 2012	non-surgical	NA	intact	not castrated	ring	120	20	2.8	0.4	20	5.3	2.4
39	Martí, 2012	non-	NA	intact	not castrated	anti GnRH vaccine	30	20	2.8	0.4	20	3.4	1.3

		surgical											
39	Martí, 2012	non-surgical	NA	intact	not castrated	anti GnRH vaccine	120	20	2.8	0.4	20	2.1	0.4
39	Martí, 2012	non-surgical	NA	ring	non-surgical	anti GnRH vaccine	30	20	3.9	1.1	20	3.4	1.3
39	Martí, 2012	non-surgical	NA	ring	non-surgical	anti GnRH vaccine	120	20	5.3	2.4	20	2.1	0.4

APÊNDICE 4: Base de dados utilizada na meta-análise sobre os efeitos da descorna em indicadores de BEA

Ganho médio diário de peso

study	author	how	med_type	group_cont	how_cont	group_tx	n_cont	mean_cont	sd_cont	n_tx	mean_tx	sd_tx
29	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	13	-190.0	93.0	15	-130.0	93.0
29	Sinclair, 2012	amputation	multi-modal	sham	not dehorned	knife + lido & meloxicam	13	-190.0	14.0	11	-200.0	14.0
29	Sinclair, 2012	amputation	anti-inflammatory	sham	not dehorned	knife + meloxicam	13	-190.0	15.0	13	-200.0	15.0
29	Sinclair, 2012	amputation	multi-modal	knife	amputation	knife + lido & meloxicam	15	-130.0	103.0	11	-200.0	103.0
29	Sinclair, 2012	amputation	multi-modal	knife	amputation	knife + lido & meloxicam	15	-130.0	77.0	13	-180.0	77.0
29	Sinclair, 2012	amputation	multi-modal	knife + meloxicam	amputation	knife + lido & meloxicam	13	-180.0	28.0	11	-200.0	28.0
30	Sinclair, 2012	amputation	anaesthetic	sham	not dehorned	knife + lido	9	120.0	6.1	9	125.0	6.1
30	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	9	120.0	140.0	9	5.0	140.0
30	Sinclair, 2012	amputation	anaesthetic	knife	amputation	knife + lido	9	5.0	146.0	9	125.0	146.0
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup	11	540.0	290.0	13	90.0	290.0
31	Sinclair, 2012	amputation	anaesthetic	sham	not dehorned	cup + Tri-Solfen	11	540.0	400.0	11	-110.0	400.0
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup & hot-iron	11	540.0	430.0	13	-130.0	430.0
31	Sinclair, 2012	amputation	anaesthetic	cup	amputation	cup + Tri-Solfen	13	90.0	290.0	11	-110.0	290.0
31	Sinclair, 2012	amputation	NA	cup	amputation	cup & hot-iron	13	90.0	330.0	13	-130.0	330.0
31	Sinclair, 2012	amputation	anaesthetic	cup & hot-iron	amputation	cup + Tri-Solfen	11	-130.0	30.0	13	-110.0	30.0

Concentração de cortisol

study	author	how	med_type	group_cont	how_cont	group_tx	time	n_ cont	mean_ cont	sd_ cont	n_ tx	mean_ tx	sd_ tx
8	Cooper et al., 1995	amputatio n	NA	sham	not dehorned	Barnes dehorner	30	6	17.8	16.05	6	61.67	49.81
16	Mellor et al., 2002	amputatio n	NA	sham	not dehorned	scoop	30	10	21,0	26.9	10	52,0	49.5
16	Mellor et al., 2002	amputatio n	NA	sham	not dehorned	scoop	120	10	16.8	13,0	10	32.5	18.3
16	Mellor et al., 2002	amputatio n	anaesthetic	sham	not dehorned	scoop + lido	30	10	21,0	26.9	10	28.2	54.2

16	Mellor et al., 2002	amputation	anaesthetic	sham	not dehorned	scoop + lido	120	10	16.8	13,0	10	9.2	30.7
16	Mellor et al., 2002	amputation	anaesthetic	scoop	amputation	scoop + lido	30	10	52,0	49.5	10	28.2	55.5
16	Mellor et al., 2002	amputation	anaesthetic	scoop	amputation	scoop + lido	120	10	32.5	18.3	10	9.2	30.7
25	Hubber et al., 2013	cautery iron	anaesthetic	sham	not dehorned	hot-iron + procaine	30	20	12,0	9.6	20	14.2	16.3
25	Hubber et al., 2013	cautery iron	anaesthetic	sham	not dehorned	hot-iron + procaine	120	20	8.4	8.7	20	10.6	9.3
25	Hubber et al., 2013	cautery iron	multi-modal	sham	not dehorned	hot-iron + flunexin & procaine	30	20	12,0	9.6	20	6.3	5.7
25	Hubber et al., 2013	cautery iron	multi-modal	sham	not dehorned	hot-iron + flunexin & procaine	120	20	8.4	8.7	20	4.8	3.9
25	Hubber et al., 2013	cautery iron	multi-modal	sham	not dehorned	hot-iron + flunexin & procaine	30	20	12,0	9.6	19	12.3	16.6
25	Hubber et al., 2013	cautery iron	multi-modal	sham	not dehorned	hot-iron + flunexin & procaine	120	20	8.4	8.7	19	8.1	10.1
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + procaine	cautery iron	hot-iron + flunexin & procaine	30	20	14.2	16.3	20	6.3	5.7
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + procaine	cautery iron	hot-iron + flunexin & procaine	120	20	10.6	9.3	20	4.8	3.9
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + procaine	cautery iron	hot-iron + flunexin & procaine	30	20	14.2	16.3	19	12.3	16.6
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + procaine	cautery iron	hot-iron + flunexin & procaine	120	20	10.6	9.3	19	8.1	10.1
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + flunexin & procaine	cautery iron	hot-iron + flunexin & procaine	30	20	6.3	5.7	19	12.3	16.6
25	Hubber et al., 2013	cautery iron	multi-modal	hot-iron + flunexin & procaine	cautery iron	hot-iron + flunexin & procaine	120	20	4.8	3.9	19	8.1	10.1
29	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	30	13	24,0	35.7	15	34.4	35.7
29	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	120	13	31.6	24.8	15	44.7	29.4
29	Sinclair, 2012	amputation	multi-modal	sham	not dehorned	knife + lido & meloxicam	30	13	24,0	35.7	15	43.8	35.7
29	Sinclair, 2012	amputation	multi-modal	sham	not dehorned	knife + lido & meloxicam	120	13	31.6	21.9	15	42.3	24.5
29	Sinclair, 2012	amputation	anti-inflammatory	sham	not dehorned	knife + meloxicam	30	13	24,0	35.7	13	41.7	35.7
29	Sinclair, 2012	amputation	anti-inflammatory	sham	not dehorned	knife + meloxicam	120	13	31.6	18.2	13	46.7	18.1
29	Sinclair, 2012	amputation	multi-modal	knife	amputation	knife + lido & meloxicam	30	15	34.4	35.7	11	43.8	35.7

29	Sinclair, 2012	amputation	multi-modal	knife	amputation	knife + lido & meloxicam	120	15	44.4	19.3	11	42.3	18.7
29	Sinclair, 2012	amputation	anti-inflammatory	knife	amputation	knife + meloxicam	30	15	34.4	35.7	13	41.7	35.7
29	Sinclair, 2012	amputation	anti-inflammatory	knife	amputation	knife + meloxicam	120	15	44.4	22.5	13	46.7	16,0
29	Sinclair, 2012	amputation	multi-modal	knife + meloxicam	amputation	knife + lido & meloxicam	30	13	41.7	35.7	11	43.8	35.7
29	Sinclair, 2012	amputation	multi-modal	knife + meloxicam	amputation	knife + lido & meloxicam	120	13	46.7	14.1	11	42.3	20.7
30	Sinclair, 2012	amputation	anaesthetic	sham	not dehorned	knife + lido	30	9	43.7	11.9	9	41.9	8.3
30	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	30	9	43.7	12.7	9	49,0	10.5
30	Sinclair, 2012	amputation	anaesthetic	knife	amputation	knife + lido	30	9	49,0	11.7	9	41.9	10.7
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup	30	11	14.6	19.7	13	29,0	19.7
31	Sinclair, 2012	amputation	anaesthetic	sham	not dehorned	cup + Tri-Solfen	30	11	14.6	19.7	11	24,0	19.7
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup & hot-iron	30	11	14.6	19.7	13	27.6	19.7
31	Sinclair, 2012	amputation	anaesthetic	cup	amputation	cup + Tri-Solfen	30	13	29,0	19.7	11	24,0	19.7
31	Sinclair, 2012	amputation	NA	cup	amputation	cup & hot-iron	30	13	29,0	19.7	13	27.6	19.7
31	Sinclair, 2012	amputation	anaesthetic	cup & hot-iron	amputation	cup + Tri-Solfen	30	13	27.6	19.7	11	24,0	19.7
32	Sinclair, 2012	amputation	NA	sham	not dehorned	scoop	30	6	17.4	12.3	10	36.3	12.3
32	Sinclair, 2012	amputation	NA	sham	not dehorned	knife	30	6	17.4	9.9	8	33.7	9.9
32	Sinclair, 2012	cautery iron	NA	sham	not dehorned	hot-iron	30	6	17.4	11,0	11	33.9	11,0
32	Sinclair, 2012	amputation	NA	scoop	amputation	knife	30	10	36.3	1.9	8	33.7	1.9

Vocalização

study	author	how	med_type	group_cont	how_cont	group_tx	n_cont	mean_cont	sd_cont	n_tx	mean_tx	sd_tx
29	Sinclair, 2012	amputation	multi-modal	knife	amputation	knife + lido & meloxicam	15	15.6	12.4	11	5.5	19.8

29	Sinclair, 2012	amputation	anti-inflammatory	knife	amputation	knife + meloxicam	15	15.6	12.4	13	17.6	11.1
29	Sinclair, 2012	amputation	multi-modal	knife + meloxicam	amputation	knife + lido & meloxicam	13	17.6	11.1	11	5.5	19.8
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup	11	2.1	4.6	13	8.1	4.3
31	Sinclair, 2012	amputation	anaesthetic	sham	not dehorned	cup + Tri-Solfen	11	2.1	4.6	11	7,0	3.7
31	Sinclair, 2012	amputation	NA	sham	not dehorned	cup & hot-iron	11	2.1	4.6	13	15.1	5.7
31	Sinclair, 2012	amputation	anaesthetic	cup	amputation	cup + Tri-Solfen	13	8.1	4.3	11	7,0	3.7
31	Sinclair, 2012	amputation	NA	cup	amputation	cup & hot-iron	13	8.1	4.3	13	15.1	5.7
31	Sinclair, 2012	amputation	anaesthetic	cup & hot-iron	amputation	cup + Tri-Solfen	13	15.1	5.7	11	7,0	3.7
32	Sinclair, 2012	amputation	NA	scoop	amputation	knife	10	10.8	7.1	8	10.6	6,0

APÊNDICE 5: Normas utilizadas para redação do Capítulo II

INSTRUCTIONS FOR AUTHORS (REVISED 2015)
Journal of Animal Science

The Instructions for Authors, Journal of Animal Science (JAS) is divided into 2 sections:

I. Manuscript Preparation, which describes the Style and Form that authors must follow in the preparation of manuscripts; and

II. Policies and Procedures of JAS, which describes the mission of JAS, contact information, care and use of animals, protection of human subjects, conflict of interest, types of articles published in JAS, manuscript submission, copyright policies, review procedures and policies, papers in press, author proofs, and publication charges.

I. MANUSCRIPT PREPARATION (STYLE AND FORM)

The most important thing authors can do as they prepare their manuscripts is to consult a recent issue of JAS to see the acceptable format for headings, title page, ABSTRACT, Key words, INTRODUCTION, MATERIALS AND METHODS, RESULTS, DISCUSSION (or combined RESULTS AND DISCUSSION), LITERATURE CITED, and tables and figures (including figure captions). Each of these topics is described in this document. The headings are shown in uppercase letters to illustrate how they should appear in manuscripts. A basic manuscript template in Microsoft Word is available at http://www.animalsciencepublications.org/publications/ jas/infora. **Manuscripts that are not consistent with the Instructions for Authors will be immediately rejected.**

General. Manuscripts must be written in English and must use American spelling and usage, as well as standard scientific usage. The following online resources provide detailed information.

• For general style and form, authors should follow that recommended in Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers. 7th ed. Council of Science Editors, Reston, VA.

• For American English spelling and usage, consult Merriam-Webster Online. http://www.m-w. com/

• For how to use numbers, refer to Policies Regarding Number Usage later in this document.

• For SI units, the National Institute of Standards and Technology provides a comprehensive guide. http://physics.nist.gov/cuu/Units/index.html

• For capitalization and spelling of plants, consult the USDA Plants website. http://plants.usda.gov

• For anatomical nomenclature, consult the current Nomina Anatomica Veterinaria. http:// www.wava-amav.org/Downloads/nav_2012.pdf

• For bacterial nomenclature, consult Approved Lists of Bacterial Names. http://www.bacterio. net/alintro.html

Manuscripts should be prepared double-spaced in Microsoft Word, with lines and pages numbered consecutively, using Times New Roman font at 12 points and no less than 2.54-cm (1 inch) margins all around. Special characters (e.g., Greek and symbols) should be inserted

using the symbols palette available in this font. Complex equations should be entered using MathType (http://www.dessci.com/en/products/mathtype/). Tables and figures should be placed in separate sections at the end of the manuscript, and not placed in the text. Manuscripts should be uploaded to Thomson Reuters ScholarOne Manuscripts (formerly called Manuscript Central) using the fewest files possible to facilitate the review and editing processes.

Manuscripts should contain the following sections in this order.

Title Page. The title page includes a running head (the first word only and any proper nouns capitalized and no more than 45 keystrokes [i.e., characters and spaces; a space is counted as a keystroke]); the title (only the first word and any proper nouns capitalized, as brief as possible, and including the species involved); names of authors (e.g., T. E. Smith; no title, positions, or degrees) and institutions, including the department, city, state or country (all with first letters capitalized), and ZIP or postal code. Author affiliations are footnoted using the symbols *, †, ‡, §, #, ‖, and ¶ and are placed below the author names. If a consortium is listed in the by line, a footnoted reference to a website showing the names and affiliations of each member of the consortium should be included in acknowledgements; names and affiliations of each member of the consortium will not be listed on the title page. Superscript numbers are used to reference footnotes on the first page. Acknowledgments, including acknowledgements of consortia, grants, experiment station, or journal series number, are given as a footnote to the title. Authors disclosing potential or actual conflicts of interest related to the research presented in the manuscript should describe this in a footnote with other acknowledgements (for details, see Conflict of Interest).

Abstract. ABSTRACT consists of no more than 2,500 keystrokes (characters and spaces) in one paragraph and contains a summary of the pertinent results, with statistical evidence (i.e., P-values), in a brief but understandable form, beginning with a clear statement of the objective and ending with the conclusions, with no references cited. Abbreviations in the abstract that are not in **Standard *JAS* Abbreviations** must be defined at first use. Key words. List up to 6 key words or phrases including the species, variables tested, and major response criteria. The first letter of each key word is lowercase, Instructions for Authors of Journal of Animal Science unless it is a proper noun; key words are separated by commas and presented in alphabetical order; and no abbreviations should be used. Because major words in the title are not used for the subject index, which is published in the last issue of each volume of JAS, appropriate words from the title should be listed as key words.

Introduction. INTRODUCTION must not exceed 2,000 keystrokes (characters and spaces) and must contain a brief justification for conducting the research, the hypotheses to be tested, and the objective(s). Extensive discussion of relevant literature should be included in DISCUSSION, not in INTRODUCTION.

Materials and Methods. MATERIALS AND METHODS is a required section and must contain a clear description or specific original reference for all biological, analytical, and statistical procedures. All modifications of procedures must be explained. Diets, dates of experimental activities if appropriate, animals (breed, sex, age, body weight, and weighing conditions [i.e., with or without restriction of feed and water]), surgical techniques, measurements, and statistical models should be described clearly and fully. Manufacturer information must be provided at the first mention of each proprietary product used in the research (for details see, **Commercial Products**). Appropriate statistical methods should be used, although the biology should be emphasized. Statistical methods commonly used in the animal sciences need not be described in detail, but adequate references should be provided. The statistical model, classes, blocks, and experimental unit must be designated. Any restrictions used in estimating parameters should be defined. Reference to a statistical package without reporting the sources of variation (classes) and other salient features of the analysis, such as covariance or

orthogonal contrasts, is not sufficient. Always reference SAS with the manufacturer information (SAS Inst. Inc., Cary, NC); do not call out as a reference in LITERATURE CITED. The threshold (e.g., $P < 0.05$) for significance should be stated. A statement of the results of the statistical analysis should justify the interpretations and conclusions. The experimental unit is the smallest unit to which an individual treatment is imposed. Measurements on the same experimental unit over time are not independent and should not be considered as independent experimental units. Provide a validation for assays (e.g., mean and CV for repeated analysis of a sample [both between and within-assay if available] and the sensitivity [minimum amount or concentration detectable]). Also, provide a publication reference for the methods used in kits. Centrifugal force should be provided in × g, not rpm, and duration and temperature of centrifugation must be included. Include volume of blood collected, container used, and amount of preservative or anticoagulant (e.g., 10 µL of heparin).

Results. RESULTS are presented in the form of tables or figures when feasible. The text should explain or elaborate on the tabular data, but numbers should not be repeated within the text. Sufficient data, all with some index of variation attached, including significance level (i.e., P-value), should be presented to allow readers to interpret the results of the experiment. Reporting the P-value is preferred to the use of the terms significant and highly significant, which are more editorial than quantitative descriptions. Thus, the P-value (e.g., $P = 0.042$ or $P < 0.05$) should be presented, thereby allowing readers to decide what to reject. Other probability (alpha) levels may be discussed if properly qualified so that the reader is not misled (e.g., trends in the data).

Discussion. DISCUSSION contains the author's, or authors', interpretations of the results of the study. The presentation should be clear and concise, address biological mechanisms and their significance, and integrate the research findings with the body of previously published literature to provide readers with a broad base on which to evaluate the author's, or authors', interpretations and assertions. Authors may speculate, but they should make it clear that their statements are speculative, rather than factual. A stand-alone DISCUSSION should not refer to any tables or figures, nor should it include P-values, unless citing a P-value from another work. The discussion must be consistent with the data from the research.

Results and Discussion. In JAS, authors have the option of combining the results and discussion into one section.

Literature Cited. To be listed in LITERATURE CITED, papers must be published or accepted for publication ("in press"). Personal communications and unpublished data must not be included in LITERATURE CITED. Guidelines and formats for references and citations are described in the Literature Cited Section of this document.

Tables and Figures. Tables and figures must be prepared so they meet the stand-alone criterion; that is, information in a table or figure can be understood without referring to information in the body of the manuscript. Tables and figures shall be placed at the end of the manuscript. Each table and each figure shall be placed on a separate page (separated with section breaks) and identified with table and figure numbers. Author-defined abbreviations must be defined (or redefined) in each table and figure. Manufacturer name and location must be provided for any proprietary product appearing in a table or figure.

Tables must be created using the table feature in MS Word (for instructions, **see Guidelines for Creating Tables Using Microsoft Word** (http://www.animalsciencepublications.org/files/publications/jas/wordtableguidelines-jas.pdf). Refer to a recent issue of JAS for examples of table construction. When possible, tables should be organized to fit across the page (i.e., portrait layout) without running broadside (i.e., landscape). Each column must have a heading (e.g., Item, Ingredient, Trait, Fatty acid). Units (e.g., kg) should be separated from headings by a comma, rather than being shown in parentheses. Limit the data field to the minimum needed for meaningful comparison

within the accuracy of the methods. In the body of the table, numerals are used to reference footnotes. Each footnote should begin on a new line. Lowercase, superscript letters are used to indicate significant differences among means within a row or column and to reference footnotes explaining how to interpret the letters.

Figures should follow the **Quality Guidelines for Journal of Animal Science (JAS) Figures** (http:// www.animalsciencepublications.org/files/pub- Instructions for Authors of Journal of Animal Science lications/jas/infora-guidelines-for-figures.pdf). Figure captions should be typed double-spaced on a separate page. Now that JAS is a fully electronic publication, authors are encouraged to use color to enhance figures; there are no additional fees for color figures and images in issues of JAS.

Individuals may purchase print-on-demand copies of JAS issues from Sheridan Press. Print-on-demand copies will contain gray-scale, rather than color, figures and images. To purchase these, contact Sheridan at Journal of Animal Science or American Society of Animal Science, PO Box 465, Hanover, PA 17331 P: 717-632-3535, F: 717-633-8920, E: pubsvc.tsp@sheridan.com.

Appendices. An appendix or appendices are optional and used to provide numerical examples or give extensive detail of analytical procedures. However, if the supplemental material is of interest only to a limited number of JAS readers, it should not be included as an appendix. Instead, state that supplemental information is available on request from the corresponding author; addresses for websites with appropriate supplemental information are acceptable. If extensive, the data may be included as an e-supplement to the manuscript (see ***E-Supplements***). Appendices should follow LITERATURE CITED and be introduced with a major heading (e.g., APPENDIX 1: TITLE).

E-Supplements. Authors may present material in an e-supplement (e.g., detailed data sets, Excel files, and video) that is more extensive or detailed than necessary for a JAS article. A note will appear in the JAS article that more material can be found online. Material in an e-supplement must undergo peer review and, thus, should be in a format that is easily accessible (i.e., does not require dedicated software or software that is not generally available) to most reviewers and readers.

Additional Usage Notes

Numbers. For details, see **Policies Regarding Number Usage *for Journal of Animal Science*** later in this document.

Abbreviations. Except to begin a sentence and when specifically contraindicated (e.g., units of time should only be abbreviated when used with a number), authors must use the abbreviations that are listed in this document under **STANDARD *JAS* ABBREVIATIONS.** Abbreviations in the text that are not listed in **STANDARD *JAS* ABBREVIATIONS** must be defined at first use, unless they are international abbreviations for elements, units of measure, amino acids, and chemicals, as examples. Abbreviations listed in **STANDARD *JAS* ABBREVIATIONS** or standard international abbreviations cannot be used to create author-defined abbreviations (e.g., t = metric ton and cannot be used as an abbreviation for time, temperature, or treatment; C = carbon and cannot be used for Control).

Once defined, author-defined abbreviations should always be used, except to begin a sentence. Author-defined abbreviations must be defined in the abstract and redefined at first use in the body of the manuscript, in each table, and in each figure. Authors should avoid excessive use of author-defined abbreviations.

Gene and Protein Names. Because there is no universally accepted style for gene and protein names that applies to all species, the JAS asks authors to assume the responsibility of using the convention appropriate for the particular species. Some general guidelines can be found in the CSE Manual for Authors, Editors, and Publishers (7th ed., 2006). For example, the

gene that codes for the protein p53 is TP53 in humans and Trp53 in mice (note that, by convention, gene names are italicized, and protein names are generally not italicized).

Quantitative Trait Loci and DNA Markers and Microarray Data. Authors of papers that contain original quantitative trait loci (QTL) or DNA marker association results for livestock are strongly encouraged to make their data available in an electronic form to one of the publicly available livestock QTL databases after the manuscript appears on the JAS First Look website (http://www.animalsciencepublications.org/publications/jas/first-look). The date on which the paper is posted to the JAS-Papers in Press website may represent the official public disclosure date for the contents of the article. Current QTL databases for livestock include, but may not be limited to, the Animal QTL database (http:// www.animalgenome.org/QTLdb) and the Bovine QTL database (http://genomes.sapac.edu.au/bovineqtl/index. html). Similarly, for microarray data we request that all authors using microarray data analysis in their research submit a complete data set to 1 of 3 databases before submission of a manuscript: the NCBI Gene Expression Omnibus (GEO; http://www.ncbi.nlm.nih.gov/projects/ geo), the EMBL-EBI ArrayExpress repository (http:// www.ebi.ac.uk/arrayexpress), or the Center for Information Biology Gene Expression (CIBEX) database.

Commercial Products. The use of names of commercial products should be minimized. When a commercial product is used as part of an experiment, the manufacturer name and location (city and state if in the US; city, administrative region or district [e.g., province], and country if outside the US) or a website address must be given parenthetically at first mention in text, tables, and figures. The generic name should be used subsequently. No ™, ®, or © symbols should be used.

General Usage.

• Abbreviations are not used to begin sentences. Words must be spelled out

• Note that “and/or” is allowed but not preferred; we ask that authors choose the more appropriate meaning or use “x or y or both” if possible.

• “Sex” should be used, rather than “gender.” Gender is more appropriate for describing a role in society than for describing biological sex.

• State total sample size (e.g., the study included a total of 600 animals), rather than using “N” to represent total sample size.

• In math, the hierarchy for brackets and parentheses is [( )]. For example, [(2 + 3) × (12 ÷ 2)] × 2 = 60.

• In writing, however, a parenthetical remark within a parenthetical is punctuated as brackets within parentheses, ([ ]). For example, “The title page includes a running head (no more than 45 keystrokes [i.e., characters plus spaces]); the title…” Instructions for Authors of Journal of Animal Science

• Meat shear force should be expressed in kilograms (kg), although newtons (N) may also be acceptable.

• Report time using the 24-h system (e.g., 1410 h rather than 2:10 p.m.).

• Use italics to designate genus and species (e.g., Bos taurus) and botanical varieties (e.g., Medicago sativa var. Potomac). Designations for botanical cultivars should be preceded by “cv.” or enclosed in single quotes (e.g., Festuca arundinacea cv. Kentucky 31 or Festuca arundinacea ‘Kentucky 31’).

• Names of muscles are not italicized.

• Specify the basis (i.e., as-fed or dry matter) for dietary ingredient and chemical composition data listed in text or in tables. Similarly, specify the basis for tissue composition data (e.g., wet or dry basis).

• Calculations of efficiency should be expressed as output divided by input (i.e., gain:feed, not feed:gain). This avoids the spurious positive and negative infinity values when body weight

gain is zero or negative. It also avoids the confusion associated with discussing an improvement as being a decrease.

• A diet is a feedstuff or a mixture of feedstuffs; a ration is the daily allotment of the diet.

• Restrict the use of “while” and “since” to meanings related to time. Appropriate substitutes include “and,” “but,” or “whereas” for “while,” and “because,” “even though,” or “although” for “since.”

• The word “Table” is capitalized and never abbreviated.

• Except to begin a sentence, the word “Figure” should be abbreviated to “Fig.”

• Except to begin a sentence, experiment and equation should be abbreviated to Exp. and Eq., respectively, when preceding a numeral (e.g., Exp. 1).

• Avoid jargon unfamiliar to scientists from other disciplines. Do not use the term “head” to refer to an animal or group of animals. Instead, use animal, sow, ewe, steer, heifer, cattle, etc.

• Avoid bi- as a prefix because of its ambiguity; biweekly means twice per week and once every 2 weeks.

• Breed and variety names should be capitalized (e.g., Landrace and Hereford).

• Trademarked or registered names should be capitalized, but no ™ or ® symbols should be used.

II. POLICIES AND PROCEDURES OF JAS

The mission of the American Society of Animal Science (ASAS) is **to “foster the discovery, sharing, and application of scientific knowledge concerning the responsible use of animals to enhance human life and well-being”** (https://asas.org/about-asas/historyand-mission). The Journal of Animal Science, which is published monthly by ASAS, accepts manuscripts presenting information for publication with this mission in mind.

The JAS is divided into the following Sections: Animal Genetics; Animal Nutrition: Nonruminant Nutrition; Animal Nutrition: Ruminant Nutrition; Animal Physiology; Animal Production; Animal Products; Special Topics; and Symposia, which contains invited manuscripts from symposia at ASAS meetings. Manuscripts that do not fit one of the JAS Sections will not be considered for publication.

The Editor-in-Chief, Managing Editor, and Section Editors establish the editorial policies of JAS, subject to review by the publications committee and ASAS Board of Directors. The views expressed in articles published in JAS represent the opinions of the author(s) and do not necessarily reflect the official policy of the institution with which an author is affiliated, the ASAS, or the JAS Editor-in-Chief. Authors are responsible for ensuring the accuracy of collection, analysis, and interpretation of data in manuscripts and ultimately for guaranteeing the veracity of the contents of articles published in JAS.

The JAS is one of the most frequently cited, peer-reviewed, agriculturally oriented research journals in the world, based on statistics published by Thomson Reuters (formerly ISI Inc.; Philadelphia, PA). Its high ranking in several categories attests to the quality standards of the JAS editors, editorial board, and staff and the authors who submit manuscripts for publication.

Contact Information

For information on the scientific content of the journal, contact the Editor-in-Chief, Dr. Gregory S. Lewis, American Society of Animal Science, P.O. Box 7410, Champaign, Illinois 61826-7410; e-mail: glewis@asas. org.

For questions about submitting a manuscript and ScholarOne Manuscripts, contact Mr. Brett Holte, Submission Services Manager; e-mail: bholte@sciencesocieties.org.

For assistance with author proofs, contact Ms. Emily Mueller, Managing Editor; e-mail: emueller@sciencesocieties.org.

Care and Use of Animals

All authors submitting to JAS must complete the Care and Use of Animals form certifying that any research that involves animals has followed established standards for the humane care and use of animals and must specify which standards were used. Only investigations that have followed high standards for the humane care and use of animals in research will be reported in JAS.

Also, the manuscript must include a statement of institutional animal care and use committee (IACUC), or equivalent, approval of all animal procedures. The IACUC statement should appear as the first item in MATERIALS AND METHODS and should specify which publically available animal care and use standards were followed (e.g., FASS Guide for the Care and Use of Agricultural Animals in Research and Teaching; Primary Industries Ministerial Council, Model code of practice for the welfare of animals: the sheep). The manuscript should describe anesthetics, analgesics, tranquilizers, and care taken to minimize pain and discomfort during preoperative, operative, and postoperative procedures. If research requires discomfort to the animals or stress- Instructions for Authors of Journal of Animal Science ful conditions, justification for these conditions must be evident in papers published in JAS.

Protection of Human Subjects

In the United States, federally funded or regulated research involving human subjects must comply with Code of Federal Regulations (CFR), Title 45 Public Welfare, Part 46 Protection of Human Subjects. However, CFR 45 Part 46.101(b) exempts some research from these regulations. For all exempted research and other details, see http://www.hhs.gov/ohrp/humansubjects/ guidance/45cfr46.html. Exempted research includes that in which the only involvement of human subjects is for “taste and food quality evaluation and consumer acceptance if 1) wholesome foods without additives are consumed or 2) a food is consumed that contains a food ingredient at or below the level and for a use found to be safe, or agricultural chemical or environmental contaminant at or below the level found to be safe, by the Food and Drug Administration or approved by the Environmental Protection Agency or the Food Safety and Inspection Service of the U.S. Department of Agriculture.” If human subjects were used in exempted research and the research was in compliance with CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must state in **MATERIALS AND METHODS** or acknowledgements that they were in full compliance. If human subjects were used in research that was not exempted in CFR 45 Part 46, or equivalent regulations where the research was conducted, authors must certify that the research received a priori approval from an appropriate Institutional Review Board.

Conflict of Interest

All JAS editors, ASAS staff, ASAS Board of Directors, and submitting authors must disclose any actual or potential conflicts of interest that may affect their ability to objectively present or review research or data. This generally includes any relevant professional, personal, political, intellectual, religious, or financial interest in, or relationship with, an individual or business that could have an actual or perceived influence, positive or negative, on the conduct and publication of the research or data. Financial relationships generally refer to financial benefits accrued to authors through avenues such as salary, consulting fees, honoraria

(including paid holidays, use of vacation property, country club privileges, and other nonmonetary rewards for service), intellectual property rights, royalties, business ownership, and investments, other than diversified mutual funds or the equivalent.

Disclosures for JAS authors are to be provided as an acknowledgement on the title page of a manuscript (for instructions, see ***Title Page***). The JAS may use such information as a basis for editorial and publication decisions, and may publish such disclosures if that is deemed relevant and sufficient. The JAS editors, ASAS staff, and ASAS Board of Directors with actual or potential conflicts of interest that may affect their ability to objectively evaluate or manage a manuscript will be prevented from gaining access to the manuscript and associated documents, unless they are an author or coauthor, in which case ScholarOne Manuscripts will limit their access to the Corresponding Author Center. When the current Editor-in-Chief, for example, has an actual or potential conflict of interest with a manuscript, a former Editor-in-Chief will assume the responsibilities of the Editor-in-Chief for that manuscript.

Types of Articles

Articles published in JAS encompass a broad range of research topics in animal production and fundamental aspects of genetics, nutrition, physiology, and preparation and utilization of animal products. Many articles are multidisciplinary and cannot be conveniently categorized. Articles typically report research with cattle, goats, pigs, and sheep. However, studies involving other farm animals (e.g., poultry and meat and working horses) and companion animals, including performance and recreational horses, aquatic, and wildlife species will be considered for publication. Studies with laboratory animal species that address fundamental questions related to the biology of livestock, companion animals, and other managed animals may be considered. Manuscripts that report research on production issues in animals other than those constituting the main focus of JAS should be submitted to other journals.

The preceding paragraph is not meant to exclude manuscripts but, rather, is a clarification of the focus of JAS. Authors may contact the Editor-in-Chief if there are questions about whether the topic of a manuscript is appropriate for JAS.

Research Articles. Results of research contained in manuscripts submitted to JAS must not have been published in or submitted previously to a peer-reviewed scientific journal. Previous presentation at a scientific meeting or the use of data in field-day reports or similar documents, including press publications or postings to personal or departmental websites, do not preclude the publication of such data in JAS. However, abstracts, proceedings papers, field-day reports, or similar presentations that are expanded to produce full-length manuscripts should be referenced and cited in JAS manuscripts. Articles simultaneously posted to websites and submitted to JAS should carry a disclaimer on the website that this version of the paper has not undergone JAS peer-review and is not to be considered the final published form of the article. If the article has been published in JAS, the author should include the complete JAS citation so that proper credit can be given to JAS as the publisher of the article. Because JAS holds the copyright to articles it publishes, posting altered JAS articles that are represented as exact duplicates of the published version constitutes copyright violation.

Review Articles. The journal publishes invited review articles. The Editor-in-Chief, in consultation with Section Editors and the ASAS Board of Directors, identifies invited reviews. Section Editors may solicit proposals for review articles to be published in JAS, after consultation with and approval by the Editor-in-Chief; the authors may be responsible for a portion of the publication charges for invited reviews. Unsolicited review Instructions for Authors of Journal of Animal Science articles will not be considered.

Special Topics. This Section includes Biographical or Historical Sketches and Contemporary Issues in the animal sciences. Even though Biographical or Historical Sketches are part of the

Special Topics Section, they will be published on the ASAS website and in the Association News section of JAS. The frequency of publication depends on the availability of the prepared sketches. For more information, see http://www.animalsciencepublications.org/publications/jas/infora..

Contemporary Issues include topics such as environmental concerns, legislative proposals, systems analysis, and various "newsworthy" scientific issues. Even though Contemporary Issues manuscripts do not have to include original data, authors' assertions should be substantiated with references to established information from credible published sources.

Special Topics papers will be subject to peer review in a manner similar to other JAS submissions. Because of the nature of these manuscripts, their format may vary from that of standard scientific articles, although ABSTRACT and INTRODUCTION must be consistent with keystroke (characters and spaces) limitations defined earlier in this document.

Teaching articles should be submitted to Natural Sciences Education, which is a joint venture of several professional societies, including the ASAS. Articles in Natural Sciences Education are "written by and for educators in extension, universities, industry, administration, and grades K–12" and highlight teaching techniques, concepts, ideas, and other teaching-related issues. The goal is build a portfolio of teaching-related articles that can be accessed at a single location. For detailed information about Natural Sciences Education, see https://www.agronomy.org/publications/nse.

Technical Notes. A technical note is used to report a new method, technique, or procedure of interest to JAS readers. When possible, a technical note should include a comparison of results from the new method with those from previous methods, using appropriate statistical tests. The advantages and disadvantages of the new procedure should be discussed. When typeset for publication, a technical note shall not exceed 8 pages (approximately 12 Microsoft Word document pages), including tables and figures. "Technical note:" shall be the first portion of the title of such manuscripts. The review process for a technical note will be the same as that for other manuscripts. Information that is more extensive or detailed than necessary for a Technical note may be presented in an e-supplement (see ***E-Supplements***). Short communications, brief communications, and similar types of articles will not be considered for publication in JAS.

Letters to the Editor. A letter judged suitable for publication will be printed in a "Letters to the Editor" section of JAS. The purpose of this section is to provide a forum for scientific exchange relating to articles published in JAS. To be acceptable for publication, a letter must adhere to the following guidelines. 1) Only a letter that addresses matters of science and relates to information published in JAS will be considered. In general, a letter should not exceed 5,000 keystrokes and should contain no more than 5 citations. 2) A letter should provide supporting evidence based on published data for the points made or must develop logical scientific hypotheses. A letter based on conjecture or unsubstantiated claims will not normally be published. No new data may be presented in a letter. 3) The Editor-inChief will evaluate each letter and determine whether a letter is appropriate for publication. If a letter is considered appropriate, the author(s) of original JAS article(s) will be invited to write a letter of response. Normally both letters will be published together. 4) All letters will be subject to acceptance and editing by the Editor-in-Chief and editing by a technical editor.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be submitted electronically through ScholarOne Manuscripts at http://mc.manuscriptcentral.com/jas. Authors with questions about using the electronic manuscript submission system or, for technological reasons, are unable to submit manuscripts electronically may contact Mr. Brett Holte (bholte@sciencesocieties.org).

Copyright Agreement

Authors shall complete the Manuscript Submission and Copyright Release form for each new manuscript submission. The form is completed during the submission process through ScholarOne Manuscripts. Authors, such as United States government employees, who are unable to grant copyright to ASAS must indicate the reason for exemption on the form; material that was produced as an official duty of a U.S. Government employee is considered public domain. The American Society of Animal Science holds the copyright to material published in JAS. Persons who wish to reproduce material in JAS must request written permission to reprint copyrighted information from the Managing Editor, Ms. Emily Mueller (emueller@sciencesocieties.org). Likewise, authors of JAS manuscripts who include material (usually tables or figures) taken from other copyrighted sources must secure permission from the copyright holders and provide evidence of this permission at the time the manuscript is submitted to JAS for review. Tables or figures reproduced from the work of others, or data extracted from the work of others and used to construct summary tables (or figures) or for meta-analyses, must include an acknowledgement of the original source in a footnote or legend and, when appropriate, a complete citation in LITERATURE CITED. The ASAS, however, grants to the author(s) of JAS articles the right of republication in any book of which he or she is author or editor, subject only to his or her giving proper credit in the book to the original JAS publication of the article by ASAS.

REVIEW OF MANUSCRIPTS

General Procedures. The Editor-in-Chief and Section Editors determine whether manuscripts are suitable for publication in JAS. All communications about a submitted manuscript should maintain confidentiality. Section Editors handle correspondence with the peer re- Instructions for Authors of Journal of Animal Science viewers and corresponding author and promptly decide whether a manuscript should be accepted, revised, or rejected. A Section Editor's decision to accept, invite revision, or reject a manuscript after peer review is based on peer-reviewer comments and recommendations and the Section Editor's own review of the manuscript. Section Editors forward document files for accepted and rejected manuscripts to the Editor-in-Chief. After acceptance, manuscript files are forwarded to the technical editors. The Editor-in-Chief is the final arbiter concerning acceptance or rejection of manuscripts submitted for publication.

Rejections. Manuscripts are rejected for 3 general reasons. 1) The substance of the manuscript may not meet JAS standards; the work may be incomplete, the evidence may not support the conclusions, the experimental approach may be poorly conceived, or the work may repeat established fact or represent no advancement of the existing knowledge. 2) Even though the work may be sound and the results valid, the paper may be better suited for publication elsewhere. 3) Manuscripts are not written clearly, concisely, and coherently, or they are not consistent with guidelines in the 2015 Instructions for Authors, Journal of Animal Science. These manuscripts may be rejected without review. Authors whose first language is not English are urged to have an editing service review their manuscripts before they are submitted to JAS. However, JAS considers the authors, and not an editing service, responsible for the content of manuscripts.

Appeals. If a manuscript is rejected, as a first course of action the author should discuss the matter with the Section Editor responsible for the manuscript. Decisions must be appealed to the Editor-in-Chief if the author(s) believe(s) that the judgment was erroneous or biased. A

letter presenting the reasons for the appeal should be sent to the Editor-in-Chief. The Editor-in-Chief will review the author's reasons, all documents related to the manuscript, and, if necessary, consult with the Section Editor responsible for the manuscript. The Editor-inChief will then decide whether to accept or deny the appeal. A rejected manuscript may be resubmitted for publication in another Section of JAS only if the Editor in-Chief recommends this action or if the Section Editor originally assigned to the manuscript has specifically recommended this action and the Editor-in-Chief has approved the transfer.

Revisions. Most manuscripts that are eventually accepted for publication are returned to the author(s) at least once for revision. All revised manuscripts must be returned to Section Editors via JAS ScholarOne Manuscripts. Authors will be permitted 15 days to revise and return manuscripts classified as Minor Revision and permitted 35 days to revise and return manuscripts classified as Major Revision. ScholarOne Manuscripts prompts reviewers to classify manuscripts as Minor Revision or Major Revision. Section Editors will use the reviewers' classifications and their own evaluations to estimate the time required for authors to respond to reviews and use that estimate during the process of classifying manuscripts. A manuscript that will clearly require more than 35 days for revisions may be rejected. However, the author will be invited to revise the manuscript, create a new submission, and reference the original manuscript tracking number (e.g., Manuscript ID E-2015-1234) in the submission letter that accompanies the new submission. Section Editors will use the original reviews and the author's responses to the original reviews to evaluate the submission. Unless the new submission contains a significant amount of new data, there should be no reason to seek new reviews.

Manuscripts that exceed the revision-option deadline will be withdrawn. Extenuating circumstances may justify the need to extend the revision-option deadline. Requests for extensions must be communicated to the Section Editor responsible for the manuscript before the revision-option expires. The Editor-in-Chief must approve extensions. As a general rule, only one short extension will be approved. The Revision Checklist for Authors is sent with requests for revision (http://www. animalsciencepublications.org/files/publications/jas/jasrevision-checklist.pdf). Authors should closely follow the Checklist.

PAPERS IN PRESS, AUTHOR PROOFS, AND PUBLICATION CHARGES

Papers in Press. To facilitate earlier disclosure of research results, accepted manuscripts will be assigned a digital object identifier (doi) and posted to the JAS First Look site (http://www.animalsciencepublications. org/publications/jas/first-look) in the form in which they are accepted. The authors bear the primary responsibility for the content of manuscripts posted to the Papers in Press site. Because articles posted to this site have not been professionally edited and typeset, and are frequently changed in response to questions from editors, they do not represent the final, published form of the manuscript. The date a complete monthly issue of JAS is posted online is the official publication date for JAS articles. However, the date on which a manuscript is posted to the JAS-Papers in Press website may represent the official public disclosure date for the contents of the article. Authors concerned about intellectual property issues, such as patents and disclosure dates, should seek legal counsel before submitting manuscripts to a scientific journal.

Author Proofs. Accepted manuscripts are forwarded to the editorial office for technical editing and typesetting. During this process, the technical editor may contact the authors for missing information or figure revisions. The manuscript is then typeset, figures reproduced,

and author proofs (also called galley proofs) prepared. Correspondence concerning the accepted manuscript should be directed to the technical editor.

Proofs of all manuscripts will be provided to the corresponding author and should be read carefully and checked against the typed manuscript. Accuracy of the author proof is the sole responsibility of the author(s). Corrections may be returned by e-mail (preferred), fax, or overnight mail. For faxed or mailed corrections, changes to the proof should be made neatly and clearly in the margins of the proof. If extensive correction is required, changes should be provided on a separate sheet of paper with a symbol indicating location on the proof. Instructions for Authors of Journal of Animal Science Changes e-mailed to the technical editor must indicate page, column, and line numbers for each correction to be made on the proof. Notes created with Adobe editing tools and pointing to specific locations for corrections may also be used. Editor queries should be answered on the galley proofs; failure to do so may delay or prevent publication. Excessive author changes made at the proof stage may result in a $250 surcharge for additional typesetting, and they may be deemed so excessive that the manuscript will be returned to the Section Editor for additional scientific review.

Publication Charges and Reprints. The journal has 2 options available for publication: open access and conventional page charges. For the open access option, authors will pay the open access fee when proofs are returned to the editorial office so that their article will become freely available upon publication in an online issue of JAS. Charges for open access publication are $2,500 per article if at least one author is a current professional member of ASAS; the charge is $3,250 when no author is a professional ASAS member. For conventional publication, the charge is $85 per printed page in JAS if at least one author is a professional ASAS member; the page charge is $170 when no author is a professional member of ASAS. Reprints may be ordered at an additional charge.

Professional membership in ASAS is available to any person who has research, educational, commercial, or administrative responsibilities or interests in the broad disciplines within animal science. Annual professional membership is $135, and the complete details are available at the following website. https://www.asas.org/ membership-services/member-information

When the author proof is sent, the author is asked to complete a reprint order form requesting the number of reprints desired and the name of the institution, agency, or individual responsible for publication charges. Now that JAS is a fully electronic publication, there are no additional charges for color figures and images that appear in electronic issues of JAS. However, authors who order reprints are responsible for paying any additional charges for printing reprints that contain color.

STANDARD *JAS* ABBREVIATIONS

The following abbreviations should be used without definition in JAS. Plural abbreviations do not contain a final “s” because the context of an abbreviation implies whether it is singular or plural. Use of the standard 3-letter abbreviations for amino acids (e.g., Ala) is acceptable in JAS. Use of the internationally recognized chemical symbols for chemical elements (e.g., P and S) is acceptable in JAS. Except for N (not italicized), which is the recognized abbreviation for nitrogen and newton (unit of force), chemical symbols for elements are reserved for elements (e.g., C is for carbon and never for control). For chemical units and abbreviations, refer to the ACS Style Guide (published by the American Chemical Society, Washington, DC).

Physical units

Item	Unit
Bq	becquerel
°C	degree Celsius
cal	calorie
Ci	curie
cM	centimorgan (spell out morgan if used without a prefix)
Da	dalton
Eq	equivalent (only can be used with a prefix; e.g., mEq)
g	gram
ha	hectare
Hz	hertz
IU	international unit
J	joule
L	liter
lx	lux
m	meter
M	molar (concentration; preferred over mol/L)
mol	mole
N	newton (N not italicized)
N	normal (concentration)
Pa	pascal
rpm	revolutions/minute (not to be used to indicate centrifugal force)
t	metric ton (1,000 kg)
V	volt
W	watt

Units of time

Item	Unit
s	second
min	minute
h	hour
d	day
wk	week
mo	month
yr	year

Statystical symbols and abbreviations

Item	Term
ANOVA	analysis of variance
CI	confidence interval
CV	coefficient of variation

df	degree(s) of freedom (spell out if used without units)
F	F-distribution (variance ratio)
LSD	least significant difference
n	sample size (used parenthetically or in footnotes; note italics)
P	probability
r	simple correlation coefficient
r^2	simple coefficient of determination
R	multiple correlation coefficient
R^2	multiple coefficient of determination
s^2	variance (sample)
SD	standard deviation (sample)
SE	standard error
SED	standard error of the differences of means
SEM	standard error of the mean
t	t-(or Student) distribution
α	probability of Type I error
ß	probability of Type II error
μ	mean (population)
σ	standard deviation (population)
σ^2	variance (population)
χ^2	chi-squared distribution

Others

Item	Term
AA	amino acid(s)
ACTH	adrenocorticotropic hormone
ADF	acid detergent fiber (assumed sequential unless designated otherwise)
ADFI	average daily feed intake (not to be confused with DMI)
ADG	average daily gain
ADIN	acid detergent insoluble nitrogen
ADL	acid detergent lignin
ADP	adenosine diphosphate
AI	artificial insemination
AIA	acid insoluble ash
ARS	Agricultural Research Service
ATP	adenosine triphosphate
avg	average (use only in tables, not in the text)
BCS	body condition score
BLUE	best linear unbiased estimate
BLUP	best linear unbiased prediction
bp	base pair
BSA	bovine serum albumin

BTA	*Bos taurus* chromosome
BW	body weight (used for live weight)
cDNA	complementary deoxyribonucleic acid
C/EBP	CAAT-enhancer binding protein
cfu	colony-forming unit
CIE	International Commission on Illumination (Commission Internationale d'Eclairage)
CLA	conjugated linoleic acid
CoA	coenzyme A
Co-EDTA	cobalt ethylenediaminetetraacetate
CP	crude protein (N × 6.25)
D	dextro-
diam.	diameter
DE	digestible energy
DEAE	(dimethylamino)ethyl (as in DEAE-cellulose)
DFD	dark, firm, and dry (meat)
DM	dry matter
DMI	dry matter intake
DNA	deoxyribonucleic acid
EBV	estimated breeding value(s)
eCG	equine chorionic gonadotropin
EDTA	ethylenediaminetetraacetic acid
EFA	essential fatty acid
EIA	enzymeimmunoassay
ELISA	enzyme-linked immunosorbent assay
EPD	expected progeny difference(s)

Eq.	Equation(s)
Exp.	experiment (always followed by a numeral)
FFA	free fatty acid(s)
FSH	follicle-stimulating hormone
GEBV	genomic estimated breeding value(s)
g	gravity
GE	gross energy
G:F	gain-to-feed ratio
GLC	gas-liquid chromatography
GLM	general linear model
GnRH	gonadotropin-releasing hormone
GH	growth hormone
GHRH	growth hormone-releasing hormone
h^2	heritability
i.m.	intramuscular
i.p.	intraperitoneal
i.v.	intravenous
hCG	human chorionic gonadotropin
HCW	hot carcass weight

HEPES	*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid
HPLC	high-performance (pressure) liquid chromatography
i.d.	inside diameter
Ig	immunoglobulin (when used to identify a specific immunoglobulin)
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor-binding protein(s)
IL	interleukin
IVDMD	in vitro dry matter disappearance
kb	kilobase(s)
KPH	kidney, pelvic, heart fat
L	levo-
LD_{50}	lethal dose 50%
LH	luteinizing hormone
LHRH	luteinizing hormone-releasing hormone
LM	longissimus muscle
ME	metabolizable energy
MP	metabolizable protein
mRNA	messenger ribonucleic acid
MUFA	monounsaturated fatty acid
NAD	nicotinamide adenine dinucleotide
NADH	reduced form of NAD
NDF	neutral detergent fiber

NDIN	neutral detergent insoluble nitrogen
NE	net energy
NE_g	net energy for gain
NE_l	net energy for lactation
NE_m	net energy for maintenance
NEFA	nonesterified fatty acid
No.	number (use only in tables, not in the text)
NPN	nonprotein nitrogen
NRC	National Research Council
o.d.	outside diameter
OIE	World Organisation for Animal Health (Office International des Epizooties)
OM	organic matter
PAGE	polyacrylamide gel electrophoresis
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
PG	prostaglandin
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
PMSG	pregnant mare's serum gonadotropin
PPAR	peroxisome proliferator-activated receptor

PSE	pale, soft, and exudative (meat)
PUFA	polyunsaturated fatty acid(s)
QTL	quantitative trait locus (loci)
RDP	ruminally degradable protein
REML	restricted maximum likelihood
RFLP	restriction fragment length polymorphism
RIA	radioimmunoassay
RNA	ribonucleic acid
RQ	respiratory quotient
RUP	ruminally undegradable protein
rRNA	ribosomal ribonucleic acid
SAS	SAS Institute Inc. (no longer stands for Statistical Analysis System)
s.c.	subcutaneous
SDS	sodium dodecyl sulfate
SFA	saturated fatty acid
SNP	single nucleotide polymorphism
spp.	species
ssp.	subspecies
SSC	*Sus scrofa* chromosome
ST	somatotropin
TDN	total digestible nutrients
TLC	thin layer chromatography
Tris	tris(hydroxymethyl)aminomethane
tRNA	transfer ribonucleic acid
TSAA	total sulfur amino acids
USDA	US Department of Agriculture
UV	ultraviolet
VFA	volatile fatty acid(s)
vol	volume
vol/vol	volume/volume (used only in parentheses)
vs.	versus
wt	weight (use only in tables, not in the text)
wt/vol	weight/volume (used only in parentheses)
wt/wt	weight/weight (used only in parentheses)

LITERATURE CITED GUIDELINES FOR *JOURNAL OF ANIMAL SCIENCE*

References in the Text. In the body of the manuscript, refer to authors as follows: Smith and Jones (1992) or Smith and Jones (1990, 1992). If the sentence structure requires the

authors' names to be included in parentheses, the proper format is (Smith and Jones, 1982; Jones, 1988a,b; Jones et al., 1992, 1993). When there are more than 2 authors of an article, the first author's name is Instructions for Authors of Journal of Animal Science followed by the abbreviation et al. More than 1 article listed in the same sentence or parentheses must be in chronological order first and alphabetical order for 2 publications in the same year. Published, peer-reviewed articles, and not abstracts, should be cited. However, if authors originally described their work in a meeting abstract, proceedings paper, field-day report, or similar presentation and then expanded the information to produce a full-length manuscript, the authors should reference and cite those reports. If the work was someone else's and originally described in an abstract, proceedings paper, field-day report, or similar presentation, the authors should determine whether the work has been expanded and published as a peer-reviewed article, and then reference and cite the peer-reviewed article.

Work that has not been accepted for publication shall be listed in the text as "J. E. Jones (institution, city, and state or country, personal communication)." The author's own unpublished work should be listed in the text as "(J. Smith, unpublished data)." Personal communications and unpublished data must not be included in the Literature Cited section.

Literature Cited Section. To be listed in LITERATURE CITED, articles must be published or accepted for publication ("in press"). In-press citations should be updated with complete information during revision or in the author proofs. In LITERATURE CITED, citations are listed alphabetically according to author(s) last name(s), and then chronologically. The year of publication follows author names. As with text references, 2 or more publications by the same author or set of authors in the same year shall be differentiated by adding lowercase letters after the date. With the exception of consortia, the names of all authors must appear in LITERATURE CITED. For consortia, authors may include, as an acknowledgement on the title page, a link to the website containing the names and locations of the members of the consortium, or they may include the names and locations of the members of the consortium in an appendix, but not in an acknowledgement on the title page. Journal names shall be abbreviated according to the conventional ISO abbreviations used by PubMed (http:// www.ncbi.nlm.nih.gov/nlmcatalog/journals). One-word titles must be spelled out. Inclusive page numbers must be provided.

Sample references are as follows:

1. Books and articles within edited books

AOAC. 1990. Official methods of analysis. 15th ed. Assoc. Off. Anal. Chem., Arlington, VA.

NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Natl. Acad. Press, Washington, DC.

Robinson, P. H., E. K. Okine, and J. J. Kennelly. 1992. Measurement of protein digestion in ruminants. In: S. Nissen, editor, Modern methods in protein nutrition and metabolism. Academic Press, San Diego, CA. p. 121–127.

2. Handbooks, technical bulletins, theses, and dissertations

Goering, H. K., and P. J. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.

Shreck, A. L., C. D. Buckner, G. E. Erickson, and T. J. Klopfenstein. 2011. Digestibility of crop residues after chemical treatment and anaerobic storage. In: 2011 Nebraska Beef Cattle Report. Rep. No. MP94. Univ. of Nebraska, Lincoln. p. 35–36.

Sigma. 1984. Total hemoglobin: Quantitative, colorimetric determination in whole blood at 530–550 nm. Tech. Bull. No. 525. rev. ed. Sigma Chemical, St. Louis, MO.

Ward, J. D. 1995. Effects of copper deficiency on performance and immune function of cattle. PhD Diss. North Carolina State Univ., Raleigh.

3. Journal articles and abstracts

Centon, J. R., G. E. Erickson, T. J. Klopfenstein, K. J. Vander Pol, and M. A. Greenquist. 2007. Effects of roughage source and level in finishing diets containing wet distillers grains on feedlot performance. J. Anim. Sci. 85(Suppl. 2):76. (Abstr.) doi:10.2527/ jas.2006-354 (NOTE: The doi is now considered part of a citation.)

Cleale, R. M., IV, R. A. Britton, T. J. Klopfenstein, M. L. Bauer, D. L. Harmon, and L. D. Satterlee. 1987a. Induced non-enzymatic browning of soybean meal. II. Ruminal escape and net portal absorption of soybean protein treated with xylose. J. Anim. Sci. 65:1319–1326. (NOTE: Articles published before circa 2005 may not have a doi.)

Perez, V. G., A. M. Waguespark, T. D. Bidner, L. L. Southern, T. M. Fakler, T. L. Ward, M. Steidinger, and J. E. Pettigrew. 2011. Additivity of effects from dietary copper and zinc on growth performance and fecal microbiotia of pigs after weaning. J. Anim. Sci. 89:414–425. doi:10.2527/jas.2010-2839

Revidatti, M. A., J. V. Delgado Bermejo, L. T. Gama, V. Landi Periati, C. Ginja, L. A. Alvarez, J. L. VegaPla, A. M. Martínez, and BioPig Consortium. 2014. Genetic characterization of local Criollo pig breeds from the Americas using microsatellite markers. J. Anim. Sci. 92:4823-4832. doi: 10.2527/jas.2014- 7848

The Bovine Hap Map Consortium. 2009. Genome-wide survey of SNP variation uncovers the genetic structure of cattle breeds. Science. 324:528-532. doi 10.1126/science.1167936

4. Conference proceedings

Bailey, E. A., J. R. Jaeger, J. W. Waggoner, G. W. Preedy, L. A. Pacheco, and K. C. Olson. 2012. Effect of weaning method on welfare and performance of beef calves during receiving. Proc. West. Sec. Amer. Soc. Anim. Sci. 63:25-29.

NMC. 1995. Summary of peer-reviewed publications on efficacy of premilking and postmilking teat disinfections published since 1980. In: Natl. Mastitis Counc. Reg. Meet. Proc., Harrisburg, PA. Natl. Mastitis Counc., Arlington, VA. p. 82–92.

Talmant, A., X. Fernandez, P. Sellier, and G. Monin. 1989. Glycolytic potential in longissimus dorsi Instructions for Authors of Journal of Animal Science muscle of Large White pigs

as measured after in vivo sampling. In: Proc. 35th Int. Congr. Meat Sci. Technol., Copenhagen, Denmark. p. 1129.

Van der Werf, J. H. J. 1990. A note on the use of conditional models to estimate additive genetic variance in selected populations. Proc. 4th World Congr. Genet. Appl. Livest. Prod., Edinburgh, Scotland XIII:476–479.

5. Electronic Publications

FDA. 2014. Approved animal drug products online (Green Book). http://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/default.htm (Accessed 26 December 2014.)

Galyean, M. L. and P. J. Defoor. 2003. Effects of roughage source and level on intake by feedlot cattle. J. Anim. Sci. 81(E. Suppl. 2):E8–E16.

Heaton, M. P., T. S. Kalbfleisch, D. T. Petrik, B. Simpson, J. W. Kijas, M. L. Clawson, C. G. Chitko-McKown, G. P. Harhay, K. A. Leymaster, and the International Sheep Genomics Consortium. 2013. Genetic testing for TMEM154 mutations associated with lentivirus susceptibility in sheep. PLoS ONE 8(2): e55490. doi:10.1371/journal.pone.0055490

POLICIES REGARDING NUMBER USAGE FOR JOURNAL OF ANIMAL SCIENCE

Number usage in JAS is consistent with the *Scientific Style and Format: The CSE Manual for Authors, Editors, and Publishers.*

• All cardinal numbers are written as numerals except when they begin a sentence or appear in a title, when 2 numerals are adjacent in a sentence (spell out the number most easily expressed in words; e.g., two 10-kg samples), or when a number is used as a figure of speech.

• Numbers less than 1 are written with a preceding (leading) zero (e.g., 0.75).

• A comma separator is used in numbers greater than 999 (e.g., 1,234 and 1,234,567).

• Numerals should be used to designate ratios and multiplication factors (e.g., 2:1 and 3-fold increase).

• Statements such as “5 times less” should be avoided because “times” means multiplied by, and the product of a positive number (multiplicand) multiplied by 5, for example, is greater, not less, than the multiplicand. The opposite is true for a negative multiplicand, but the notion of “5 times less than –5,” for example, may be not be clear to readers.

• If a number is spelled out at the beginning of a sentence, its associated unit is also spelled out (e.g., Ten microliters of fluid . . ., not Ten μL of fluid . . .).

• Units of measurement not associated with a number should be spelled out rather than abbreviated (e.g., lysine content was measured in milligrams per kilogram of diet) unless used parenthetically, as “lysine content (mg/kg of diet) was measured,” or in tables and figures.

• Single-digit ordinals are spelled out (i.e., first through ninth); larger ordinals are expressed in numeric form. Single-digit ordinals may be expressed numerically when they form part of a series (e.g., 1st, 3rd, 10th, 20th, not first, third, 10th, and 20th).

• Measures must be presented in the metric system (SI or Système International d'Unités; see http:// physics.nist.gov/cuu/Units/introduction.html.

• When a term must be expressed in nonmetric units for clarity (e.g., bushel weight), show the nonmetric value in parentheses immediately after the metric value.

• Use "to" instead of a hyphen to indicate a numerical range in text (e.g., 1 to 10).

• Avoid the use of multiplying factors (e.g., × 10–6) in table columns or rows, or in figure axis labels because of the uncertainty about whether the data are to be, or already have been, multiplied by the factor.

• Avoid ambiguity by stating units (e.g., numbers of spermatozoa, millions/mL).

• Do not use more than one slant line (for "per") in a single expression; for example, use 5 mg/(g · d) or 5 mg · g–1 · d–1 instead of 5 mg/g/d. Mathematically, "per" implies division; when 2 "per" occur consecutively, it is unclear precisely what is being divided by what.

• Dietary energy may be expressed in calories or in joules, although joule is the standard SI unit for energy.

• Hyphenate units of measure used as preceding adjectives (e.g., 5-kg sample). Hyphens are not used with percent or degree signs.

• Insert spaces around all signs (except slant lines) of operation when these signs occur between 2 values (e.g., 10 ± 1; 5 < 10; 2 + 2 = 4).

• Convert "mg %" to other units, such as mg/L or mg/mL.

• Use "mol/100 mol" rather than "molar percent."

APÊNDICE 6: Normas utilizadas para redação do Capítulo III

Author Instructions

All manuscripts should be submitted via ScholarOne Manuscripts.

Animal Production Science welcomes the submission of articles presenting original and significant research that are within the journal´s scope.

Journal policy and scope

Research papers in **Animal Production Science** focus on improving livestock and food production, and on the social and economic issues that influence primary producers. The journal is predominantly concerned with domesticated animals (beef cattle, dairy cows, sheep, pigs, goats and poultry); however, contributions on horses and wild animals may be published where relevant. **Animal Production Science** publishes original research papers, critical review articles, and viewpoints; it does not publish technical and research notes, or short communications.

High quality original contributions are encouraged on:

- animal breeding and genetics

- animal nutrition and reproduction
- livestock farming systems, sustainability and natural resource management
- meat science and consumer acceptability
- behaviour, health and welfare
- feed quality and nutritional value
- bio-pharmaceuticals derived from animals

The subject scope extends from the molecular level through to the role of animals in farming systems. The target readership is animal scientists, and administrators and policy-makers who interface with this discipline.

Return to Index

Review papers

Prestigious, invited reviews are commissioned from authors who are world leaders in the animal sciences. Reviews should summarise a body of knowledge and, from it, formulate ideas and recommendations which would be useful to international research community. If you are interested in preparing a Review article, please discuss the subject matter with the Editor-in-Chief or the appropriate Associate Editor.

Return to Index

Perspective

A perspective is a pithy (but balanced) opinion piece about current or future directions in animal science. A perspective can critically assess current scientific topics or report on future issues that may arise from the discipline. The intent is to stimulate discussion and possible rethinking of current views in the animal sciences. Perspectives that address interdisciplinary research areas with relevance to a broader audience are of particular interest to the Editors. The Perspective should be accompanied by an abstract and generally range from 1000 to 4000 words; tables and figures can be included.

Return to Index

Editorials

Editorials are usually commissioned. Editorials are opinion pieces which reflect on papers previously or currently published in **Animal Production Science**, or on issues of general interest to the animal sciences community. They should be written in a crisp, lively style. They should have a maximum of 800 words, and not more than 5 references.

Return to Index

Comment papers

A brief comment or critique on a paper recently published in **Animal Production Science**. No abstract required. Authors of the original paper will be invited to submit a response.

Return to Index

Licence to publish

Submission of a paper is taken to mean that the results reported have not been published and are not being considered for publication elsewhere. A summary of the findings in the proceedings of a conference or in an extension article is not necessarily regarded as prior publication. However, if substantial parts of the data, such as those in Tables and Figures, have been published before, the inclusion of extra peripheral data does not alter the judgment that the paper is not new. The Editor assumes that all authors of a multi-authored paper have agreed to its submission. For details regarding copyright, please see Copyright/Licence to Publish.

Return to Index

Open access

Authors may choose to publish their paper Open Access on payment of a publication fee. See Open Access for more details.

Return to Index

Citing personal communications and statistical software

Citation of submitted manuscripts, unpublished data and personal communications should be avoided but if essential, they should be cited parenthetically in the text thus (e.g. PA Smith, pers. comm.). In such cases, the authors **must obtain permission** from the data owner to quote his or her unpublished work. Likewise, any statistical software used to process your data should be cited in brackets in the text, providing the name and version of the package and the name, city, state and country of the company that produced it.

Return to Index

Animal experimentation

Experiments involving animals are expected to have been conducted in accordance with the guidelines set out in the joint publication of the National Health and Medical Research Council of Australia, CSIRO and the Australian Agricultural Council entitled ´Code of Practice for the Care and Use of Animals for Experimental Purposes´ (National Health and Medical Research Council: Canberra, 1997). Editors will take account of animal welfare issues and reserve the right not to publish.

Return to Index

Preparing your manuscript

All authors should read at least one book on scientific writing. The titles of some suitable books are listed at the end of these notes. The work should be presented concisely and clearly in English. Introductory material, including a review of the literature, should not exceed that necessary to indicate the reason for the work and the essential background. However, a short statement explaining the broader relevance of the study can be helpful to readers. Sufficient experimental detail should be given to enable the work to be repeated, and the discussion should focus on the significance of the results. Poorly prepared or unnecessarily lengthy manuscripts have less prospect of being accepted. Authors should note the layout of headings, references, Tables and Figures in the latest issues of the Journal and follow the Journal style. Strict observance of these and the following requirements will shorten the interval between submission and publication.

Return to Index

Title

The title should be concise and informative and contain all keywords necessary to facilitate retrieval by modern searching techniques. Additional keywords not already contained in the title or abstract may be listed beneath the abstract. A short title of less than 50 letter spaces, to be used as a running head at the top of the printed page, should be supplied. The title, author(s), address(es) and short title should comprise a separate title page.

Return to Index

Summary text for the Table of Contents

This is a three-sentence paragraph of 50 to 80 words written for interested non-experts, such as journalists, teachers, government workers, etc. The text should be free from scientific jargon, and written at the level of an article in a science magazine. Your first sentence should engage the reader, convincing them that this is an important area. The second sentence should introduce the problem addressed in the paper, and state your main discovery. The final sentence should describe how the results fit into the bigger picture (i.e. implications or impact of the discovery).

Return to Index

Abstract

The abstract (preferably less than 250 words) should state concisely the scope of the work and the principal findings and should not just recapitulate the results. It should be complete enough for direct use by abstracting services. Acronyms and references should be avoided.

Please suggest 3-6 keywords, noting that all words in the title and abstract are already considered to be keywords. Keyword should list alternative spellings, e.g. defense for defence, aluminum for aluminium etc.

Return to Index

References

References are cited by the author and date (Harvard system); they are not numbered. All references in the text must be listed at the end of the paper, with the names of authors arranged alphabetically; all entries in this list must correspond to references in the text. In the text, the names of 2 co-authors are linked by ´and´; for 3 or more, the first author´s name is followed by ´*et al.*´. Where more than one reference is cited in the text, they should be listed chronologically. No editorial responsibility can be taken for the accuracy of the references. The titles of papers and the first and last page numbers must be included for all references. Papers that have not been accepted for publication cannot be included in the list of references and must be cited in the text as ´unpublished data´ or ´personal communication´; the use of such citations is discouraged. Authors should refer to the latest issues of the Journal for the style used in citing references in books and other literature. Full titles of periodicals must be given.

Examples of common references can be found in the ´Style guide for references´.

Use of referencing software. To obtain the style file for this journal, please go to the following websites.

If using ´Reference Manager´, visit http://www.refman.com/support/rmoutputstyles.asp.

If using ´ProCite´, visit http://www.procite.com/support/pcoutputstyles.asp.

If using ´EndNote*´ software, visit http://www.endnote.com/support/enstyles.asp.

*You will find the style file under the ´Agriculture´ category, listed as Animal Production Science.

Return to Index

Units

The SI system of units should be used for exact measurements of physical quantities and, where appropriate, elsewhere. The double solidus must not be used in complex groupings of units (i.e. use mg/sheep.day, not mg/sheep/day or mg sheep^{-1} day^{-1}). This Journal uses the abbreviation ´L´ for litre; ´mL´ for millilitre. When using non-standard abbreviations, define the abbreviation where it first occurs in the text.

Spell out numbers lower than 10 unless accompanied by a unit, e.g. 2 mm, 15 mm, two plants, 15 plants, but 2 out of 15 plants. Do not leave a space between a numeral and %, ‰ or ^{o}C.

Return to Index

Mathematical formulae

Formulae should be carefully typed with symbols correctly aligned and adequately spaced. If special symbols must be hand-written, they should be inserted with care and identified by pencilled notes in the margin. Judicious use should be made of the solidus to avoid 2 mathematical expressions wherever possible and especially in the running

text. Each long formula should be displayed on a separate line with at least 1 line of space above and below.

Return to Index

Tables

Tables must be numbered with Arabic numerals and each must be accompanied by a title. A headnote containing material relevant to the whole Table should start on a new line.

Tables should be arranged with regard to the dimensions of the Journal columns (8 by 21 cm), and the number of columns in the Table should be kept to a minimum. Excessive subdivision of column headings is undesirable and long headings should be avoided by the use of explanatory notes which should be incorporated into the headnote. The first letter, only, of headings should be capitalised.

The symbol of unit of measurement should be placed in parentheses beneath the column heading. The prefixes for units should be chosen to avoid an excessive number of digits in the body of the Table or a scaling factor should be added to the heading. Footnotes should be kept to a minimum and be reserved for specific items in the columns.

Horizontal rules should be inserted only above and below column headings and at the foot of the Table. Vertical rules should not be used. Each Table must be referred to in the text, and the preferred position of the Table in the text should be indicated by a note in the margin.

Short tables can frequently be incorporated into the text as a sentence or as a brief untitled tabulation. Only in exceptional circumstances will the presentation of essentially the same data in both a Table and a Figure be permitted: where adequate, the Figure should be used.

Return to Index

Figures and computer graphics

Lettering should be in sans-serif type (**Helvetica or Arial type 1 font**) with the first letter of the first word and proper names capitalised. The x-height after reduction should be 1.2-1.3 mm. Thus for the preferred reductions of graphs to 30, 40 or 50% of linear dimensions, the initial x-height of lettering should be 4, 3 or 2.5 mm respectively. Symbols and grid marks should be the same respective sizes, and curves and axes should then be either 0.8, 0.7 or 0.6 mm thick respectively. Proportionally smaller sizes of type, symbols, grid marks and curve thicknesses should be used for lesser reductions. The following symbols are readily available and should be used: ■ □ ◆ ◇ ● ○ ▲ △ ▼ ▽ ★ ☆ . The symbols + or × should be avoided. Explanations of symbols should be given in the caption to the figure, and lettering of graphs should be kept to a minimum. If information is given in a caption instead of a legend describe the lines and symbols in words (e.g. solid lines, dashed lines, dot-and-dash lines, open circles, solid circles, striped bars, cross-hatched bars and so forth).

Return to Index

Photographs

Photographs must be of the highest quality, with a full range of tones and of good contrast. Before being mounted, photographs must be trimmed squarely to exclude features not relevant to the paper and be separated from neighbouring photographs by uniform spaces that will be 2 mm wide after reduction. Lettering should be in a transfer lettering sans-serif type (**Helvetica font**) and contrast with its background; thus, white lettering should be used on dark backgrounds. The size of lettering should be such that the x-height after reduction is 1.5-l2 mm. A scale bar must be inserted on each photomicrograph and electron micrograph. Important features to which attention has been drawn in the text should be indicated (i.e. by coded upper case letters and/or arrows). Colour photographs will be accepted if they are essential, but the cost of production must be borne by the author.

Return to Index

Statistical evaluation of results

Manuscripts must contain a clear and concise description of the experimental design used; with sufficient detail such that, in the case where analysis of variance or regression models are to be used in the statistical evaluation, the reader is quite clear as to how the error term was estimated. The statistical tests should be briefly described and, if necessary, supported by references. Numbers of individuals, mean values and measures of variability should be stated. It should be made clear whether the standard deviation or the standard error has been given.

Return to Index

Nomenclature

The nomenclature of compounds such as amino acids, carbohydrates, lipids, steroids and vitamins should follow the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature. Other biologically active compounds, such as metabolic inhibitors, plant growth regulators and buffers should be referred to once by their correct chemical name (which is in accordance with IUPAC Rules of Chemical Nomenclature) and then by their most widely accepted common name. For pesticides, the latest issue of ´Pesticides - Synonyms and Chemical Names´ (Australian Government Publishing Service: Canberra) should be followed. Where there is no common name, trade names or letter abbreviations of the chemical may be used. The first letter of a trade name must be capitalised.

Return to Index

Submission of research manuscripts

To submit your paper, please use our online journal management system ScholarOne Manuscripts, which can be reached directly through this link or from the link on the journal´s homepage. If a first-time user, register via the ´Register here´ link, or use your

existing username and password to log in. Then click on the ´Author Centre´ link and proceed.

A covering letter must accompany the submission and should include the name, address, fax and telephone numbers, and email address of the corresponding author. The letter should also contain a statement justifying why the work should be considered for publication in the journal, and that the manuscript has not been published or simultaneously submitted for publication elsewhere. Suggestions of possible referees are welcome.

Return to Index

Post acceptance of manuscript

When asked to submit production files, please provide the Production Editor with the original figure files separately from the manuscript, and in highest resolution.

Ensure that figures are in their original file format (i.e. Photoshop, Adobe Illustrator, Excel, CorelDraw, SigmaPlot, etc.) rather than embedded in a Word document or converted to a derived format. However, if your figures are in a format that we do not accept, high-quality high-resolution PostScript or PDF files are acceptable. Sending files in more than one format is fine; we will use the format that will reproduce the best.

Scanned photographs must be saved as .tif files; all supplied .tif files must be compatible with Adobe Photoshop, which is the preferred program. If figures are prepared in a ´paint´ program, line art should be saved at 600 dpi, and greyscale or colour images should be saved at 300 dpi. Electronic photographic work should be submitted at the intended print size (85 mm wide for one column and up to a page width of 175 mm) (on CD-ROM if necessary). These will be returned after use if requested at the time of submission.

Colour photographs will be accepted if they are essential but the cost of colour reproduction on the printed copy must be borne by the author. The Production Editor will provide an estimate of the cost with the page proofs. Colour figures must be supplied in CMYK, not RGB, format.

Return to Index

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Return to Index

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Journal article

Hubick KT, Farquhar GD, Shorter R (1986) Correlation between water-use efficiency and carbon isotope discrimination in diverse peanut (*Arachis*) germplasm. *Australian Journal of Plant Physiology* **13**, 803-816.

Wagner TE (1985) The role of gene transfer in animal agriculture and biotechnology. *Canadian Journal of Animal Science***65**, 539-552.

Lodge GM, Murphy SR, Harden S (2003*a*) Effects of grazing and management on herbage mass, persistence, animal production and soil water content of native pastures. 1. A redgrass-wallaby grass pasture, Barraba, North-West Slopes New South Wales. *Australian Journal of Experimental Agriculture* **43**, 875-890.

Lodge GM, Murphy SR, Harden S (2003*b*) Effects of grazing and management on herbage mass, persistence, animal production and soil water content of native pastures. 2. A mixed native pasture, Manilla, North-West Slopes New South Wales. *Australian Journal of Experimental Agriculture* **43**, 891-905.

Book chapter

Blackmore DJ (1996) Are rural land practices a threat to the environment? In ´Soil science - raising the profile´. (Ed. N Uren) pp. 22-30. (ASSSI and NZSSS: Melbourne) Wolanski E, Mazda Y, Ridd P (1992) Mangrove hydrodynamics. In ´Tropical mangrove

ecosystems´. (Eds AI Robertson, DM Alongi) pp. 43-62. (American Geophysical Union: Washington DC)

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Lucas GB (1963) ´Diseases of tobacco.´ (University of North Carolina: Raleigh, NC) Attiwill PM, Adams MA (Eds) (1996) ´Nutrition of eucalypts.´ (CSIRO Publishing: Melbourne)

Hogan B, Beddington R, Constantine F, Lacy E (Eds) (1994) ´Manipulating the mouse embryo - a laboratory manual (2nd edn).´ (Cold Spring Harbor Laboratory Press: Cold Spring Harbor, NY)

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Silver MW (1970) ´An experimental approach to the taxonomy of the genus Enteromorpha (L.) Link.´ PhD thesis, University of Liverpool, UK. Harrison AJ (1961) ´Annual reproductive cycles in the Tasmanian scallop Notovola meridionalis.´ BSc (Hons) thesis, The University of Tasmania, Australia.

Report or Bulletin

Lea HW (1957) Report on a visit to the USA and Canada, April 1 to October 2, 1957. NSW Department of Agriculture, Orange, NSW.

Chippendale GM, Wolf L (1981) The natural distribution of Eucalyptus in Australia. Australian National Parks and Wildlife Service, Special Publication No. 6, Canberra. Australian Bureau of Statistics (2000) Australian Demographic Statistics, March Quarter 2000. Cat. No. 3101.0 (ABS: Canberra)

Commonwealth of Australia (1999) National Greenhouse Response Strategy. (AGPS: Canberra)

Conference Proceedings

Hayman PT, Collett IJ (1996) Estimating soil water: to kick, to stick, to core or computer? In ´Proceedings of the 8th Australian agronomy conference´. (Ed. M Asghar) p. 664. (The Australian Society of Agronomy Inc.: Toowoomba, Qld) Kawasu T, Doi K, Ohta T, Shinohara Y, Ito K (1990) Transformation of eucalypts (Eucalyptus saligna) using electroporation. In ´Proceedings of the VIIth international congress on plant tissue and cell culture´. pp. 64-68. (Amsterdam IAPTC: Amsterdam)

Simpson RJ, Bond WJ, Cresswell HP, Paydar Z, Clark SG, Moore AD, Alcock DJ, Donnelly JR, Freer M, Keating BA, Huth NI, Snow VO (1998) A strategic assessment of sustainability of grazed pasture systems in terms of their water balance. In ´Proceedings of the 9th Australian agronomy conference´. (Eds DL Michalk, JE Pratley) pp. 239-242. (The Australian Agronomy Society Inc.: Melbourne)

Online sources

Give the author, year and title and then give further information as for a chapter or journal article, but adding the essential on-line address URL and the date the information was posted or accessed (or when the address was last verified).

De Vries FP, Jansen M, Metslaar K (1995) Newsletter of agro-ecosystems modelling [Online]. November edition. Available by e-mail Listserv (camase-1@hern.nic.surfnet.nl) or Web link to gopher archives (http://www.bib.wau.nl/camase/cam-news.html) (verified 1 November 1996)

Downing MD, Langseth R, Stoffel R, Kroll T (1996) Large-scale hybrid poplar production economics: 1995 Alexandria Minnesota, establishment cost and management [Online]. In: ´Bioenergy 1996´. Proceedings of the 7th national bioenergy conference in Nashville, TN. 15-20 September, 1996. Available athttp://www.esd.ornl.gov/bfdp/papers/bioen96/downing.html. (posted 10 December 1996; verified 24 November 1998)

National Agricultural Statistics Service (1997) Crops country salinity data [Online]. Available at:http://usda.mannlib.cornell.edu/data-sets/crops/9X100 (verified 30 November 1998)

University of California (1996) Tomato pest management guidelines. University of California Pest Management Guidelines, Publication 154. [Online] (Available on-line with updates at http://www.ipm.ucdavis.edu/PMG/selectnewpest.tomatoes.html) (verified 30 November 1998)

Return to Index

Journal Style

Typing the manuscript to Journal style

1. Type with double-spacing

Manuscripts should be typed (unjustified) with *double-spacing throughout*, and with a margin of at least 3 cm on the left-hand side: A4-sized paper is preferred. All pages should be numbered consecutively. If the typescripts are produced by word processor, a good quality printer should be used. If possible, do not justify the right-hand margin and type on paper with numbered lines.

2. Title page

The first page of the manuscript should contain the title of the paper, the names and full address(es) of the author(s), and a suggested short title (less than 50 letter-spaces). All lines should be typed flush left, with superscripts (^{A}, ^{B}, ^{C}, etc.) relating the authors to their addresses.

3. Headings

All headings, including the title of the paper, should be typed in lower-case letters, with only the first letter of the first word and proper names capitalised. ´Abstract´ is a run-on

heading followed by a full stop. Main headings (Introduction, Results, etc.) should be typed **bold** or have a wavy underline or no underline at all. Second-order headings should be typed in *italics*or have a single underline. Third-order headings are run-on headings, indented, in *italics* or with a single underline, and followed by a full stop.

4. Paragraphs

All paragraphs following a heading are indented.

5. Dates

Dates in the text must be in the style 26 January 1999. In Tables, use 26 Jan. or 26.i.99.

6. Abbreviations

The following are some standard abbreviations that need not be defined: l.s.d., s.e., s.d., n.s., DM, EDTA, DNA.

7. Units

Use kg/ha not kg ha^{-1}. Also use kg/sheep.day, but not kg/sheep/day or kg $sheep^{-1}$ day^{-1}. Use the specific term such as lamb, ewe, sheep or steer rather than head or beast. Abbreviation for litre is ´L´; millilitre is ´mL´.

8. Numerals

Use numerals in the running text, except at the start of a sentence; but in titles and headings spell out numbers from 1 to 9.

9. Tables

In the text, use capital ´T´ for Table 1. Indicate the best placement of Tables by typing (Insert Table 1 here) in the margin.

Do not crowd Tables to fit on one page; use a second page if necessary. Use double-spacing for titles, headnotes and footnotes, and do not underline them. ´Table 1´ is part of the title and should not be typed on a separate line. Headnotes (or subheadings) should be used for notes or explanations that refer to the whole Table - they should be typed on a new line below the title. In column headings, side headings and Table entries, only capitalise the first letter of the first word and proper names. Units (cm, %, etc.) should be in parentheses and placed just after or below the headings (but above the line for column headings). Footnotes in Tables refer to specific column or row headings or to specific values in a Table. Use superscripts (A, B, C, etc.) for Table footnotes. Do not use vertical rules in Tables.

10. Figures

In the text and in the captions, use the abbreviated style of Fig. 1 (not Figure 1). Indicate the best placement of Figures by typing a note in the margin or between paragraphs in the text.

Captions to Figures should be typed on a separate page placed after the Tables. All lines should be typed flush left.

11. Mathematical formulae

These should be carefully typed with symbols in correct alignment and adequately spaced. If special symbols must be hand-written, they should be inserted with care and

identified by pencilled notes in the margin. Each long formula should be displayed on a separate line with at least one line of space above and below.

12. Footnotes

Footnotes should be typed within horizontal rules immediately after the text to which they refer. Footnotes should be marked with superscripts ([A], [B], [C], etc.).

13. References

Spell out the names of all periodicals, publishers, conference proceedings and books in full.

Examples of common references can be found in the Style guide for references.

Use of referencing software. To obtain the style file for this journal, please go to the following websites.

If using ´Reference Manager´, visit http://www.refman.com/support/rmoutputstyles.asp.

If using ´ProCite´, visit http://www.procite.com/support/pcoutputstyles.asp.

If using ´EndNote´ software, visit http://www.crandon.com.au.

You will find the style file under the ´Biosciences´ category, listed as *Animal Production Science* (continuing *Australian Journal of Experimental Agriculture*).

4. VITA

Maria Eugênia Andrighetto Canozzi, filha de Sérgio Canozzi e Denise Corbetta Andrighetto, é brasileira, nascida em Porto Alegre, capital do Estado do Rio Grande do Sul, no dia 27 de outubro de 1984.

De 1991 a 2000 estudou no Colégio Americano, na sua cidade natal, tendo cursado o último ano do ensino médio, em 2001, no Grupo Integrado Magdalena Kahn (GIMK), na cidade do Rio de Janeiro.

Entre 2004 e 2009, cursou Medicina Veterinária, na Universidade Federal do Rio Grande do Sul (UFRGS), sendo ou bolsista de Extensão ou de Iniciação Científica. Concluiu a graduação em julho de 2009, com a monografia intitulada “Rastreabilidade como ferramenta de certificação: uma análise do caso brasileiro”.

Em janeiro de 2013, orientada pelo Júlio Otávio Jardim Barcellos obteve o título de Mestre em Zootecnia pela UFRGS, como bolsista CAPES, com a dissertação “Metodologia para avaliação de protocolos de certificação aplicáveis na bovinocultura de corte”.

Em março de 2013, iniciou o curso de doutorado no mesmo Programa de Pós-graduação, como bolsista CNPq e sob orientação de Júlio Otávio Jardim Barcellos. Contemplada pelo programa de doutorado sanduíche no exterior (PDSE-CAPES), entre março de 2014 e fevereiro de 2015, estudou na *Universitàt Autonoma de Barcelona* (UAB) e no *Scotland’s Rural College* (SRUC), sob supervisão de Xavier Manteca Vilanova e Simon Tuner, respectivamente.