

# Welfare indicators for stunning versus non-stunning slaughter in sheep and cattle: A scoping review

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#### Abstract

Background: While most cattle and sheep in the United Kingdom are stunned before slaughter, non-stun methods are permitted to supply meat to specific consumers. This study aimed to identify the existing literature that compared animal welfare indicators during stun and non-stun slaughter, using a scoping review framework.

Method: Following a structured search strategy, including the establishment of a PICO (population, intervention, comparator and outcomes) question, a comprehensive literature search of the CAB Abstracts, MEDLINE and PubMed databases, was conducted. A total of 962 papers were identified, of which 16 were selected for data extraction.

Results: Fourteen papers concluded that welfare at slaughter was negatively impacted at non-stun slaughter in comparison to stun slaughter; two papers were inconclusive. Welfare indicators identified included biochemical parameters, brain activity and visual signs of consciousness. Limitations regarding inconsistent restraint method, neck cut position and non-standardised measures of welfare at slaughter are highlighted.

Conclusions: This study provides further evidence that stunning is an effective method to improve the welfare of sheep and cattle at slaughter. Animal welfare advisors, politicians, religious communities and others interested in animal welfare could use the findings to further discuss and establish new dialogues for producing updated guidance on animal welfare at slaughter.

#### **KEYWORDS**

animal welfare, halal, kosher, religious slaughter, shechita, stun slaughter

## **INTRODUCTION**

Slaughtering is defined as the killing of animals, especially for food intended for human consumption.<sup>1</sup> Current European Union (EU) and United Kingdom regulations state that the slaughter of animals must involve prior stunning to induce a lack of consciousness at time of killing, with the exception of slaughter by a religious method.<sup>2,3</sup>

Slaughter with prior stunning occurs in the United Kingdom in sheep and cattle following approved stunning methods. Simple stunning renders the animal temporarily unconscious and insensible to pain, distress, fear and suffering.<sup>4-6</sup> Simple stunning is reversible and does not kill the animal, so must be followed by a procedure to ensure prompt death such

as bleeding.<sup>2,3</sup> The simple stunning method most commonly used in cattle in the United Kingdom is the penetrative captive bolt gun, which applies force to the animal's skull to induce unconciousness.<sup>3</sup> Electrical head-only stunning, where an electric current is passed through the brain to generate an epileptic state, is the most common method for simple stunning in sheep in the United Kingdom.<sup>3,6</sup> Other simple stunning methods include non-penetrative captive bolt gun for animals under 10 kg.<sup>2,3</sup> Electrical head-to-back stunning, where the heart is stopped alongside loss of consciousness, or shooting with a free-bullet firearm are stun-kill methods used to slaughter ruminants in the United Kingdom.<sup>6</sup>

In the United Kingdom, non-stun methods are limited to halal or shechita slaughter.<sup>6</sup> With these

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methods, death occurs following a single ventral-neck incision, which progressively causes loss of cerebral blood supply and consciousness.<sup>7</sup> Shechita slaughter requires an uninjured animal at the time of slaughter and hence all stunning methods are forbidden.<sup>8</sup> In contrast, halal slaughter must be performed on a healthy, live animal, which theoretically permits the use of reversible stunning techniques.<sup>9</sup> A survey completed by nine halal certification bodies found that the majority allowed pre-slaughter stunning, while still operating under a written halal standard.<sup>10</sup> The United Kingdom law outlines additional regulations for religious slaughter of animals, to avoid unnecessary suffering in the absence of stunning.<sup>2</sup> Animals are restrained in an upright position and slaughtered with uninterrupted cutting movements using a sharp knife of adequate size.<sup>2</sup> In addition to the certificate of competence (CoC) and license from the Food Standards Agency (FSA), a further licence from the Rabbinical Commission for the Licensing of Shochetim is required to perform shechita.<sup>2</sup>

The growing global market for non-stun meat, especially halal, has attracted interest from a range of stakeholders, including governments, meat scientists and animal welfare activists. While shechita slaughter constitutes less than 0.5% of religious slaughter of cattle and sheep, halal methods account for 71% of all sheep slaughtered in the United Kingdom.<sup>6</sup> The majority (65%) of halal slaughter involves stunning before slaughter.<sup>6</sup> However, due to the high volumes of halal meat produced, the remaining 35% equates to approximately 60,000 sheep and 600 cattle slaughtered weekly without pre-slaughter stunning.<sup>6,11</sup> Opinions within Islamic communities on the interpretation and application of Islamic laws regarding halal meat production vary.<sup>12</sup> A recent study found that 69.9% of halal consumers indicated their preference for meat that had not been stunned.<sup>13</sup> Another study reported that 53% of halal consumers versus 95% of Islamic scholars would consider the meat of stunned animals alive at the time of slaughter to be halal.<sup>14</sup> Further discussion of the compatibility between stunning and Islamic law as defined in the Quran has been indicated as a vital next step in uniting the differing opinions within Islamic communities in the United Kingdom.<sup>14</sup>

Some studies conclude that the shechita ventralneck cut is a painless slaughter method, which renders the animal instantly insensible.<sup>15–17</sup> In contrast, a ventral-neck incision without prior stunning has been shown to cause electroencephalogram (EEG) changes that were qualitatively and quantitatively similar to those observed following scoop dehorning,<sup>18</sup> and hence has strong potential to induce pain in conscious animals. Additionally, postponement in the onset of insensibility after non-stun slaughter is reported in various studies.<sup>5,19,20</sup> The time to the onset of insensibility is further extended by a higher prevalence of false aneurysms in non-stunned animals, compared with stunned animals.<sup>21</sup> Additionally, it is a legal requirement for conventional and religious slaughter practice that animals are individually

restrained, which involves separation from the group. This may introduce an additional stress factor in herd species such as sheep and cattle.<sup>3,22,23</sup>

The published research regarding welfare at slaughter most commonly measures blood biochemical markers of stress during slaughter, such as plasma cortisol, catecholamines and lactate.<sup>18,24–27</sup> Other studies measure observed signs of unconsciousness such as loss of posture, palpebral and corneal reflexes and absence of rhythmic breathing to indicate welfare.<sup>28,29</sup> EEG or electrocorticographic (ECoG) traces are used experimentally to measure trends in electrical brain activity of anaesthetised animals, which may indicate onset and extent of insensibility following slaughter.<sup>18,30</sup> A universally preferred measurable welfare indicator at slaughter is difficult to establish, leading stakeholders to incorporate the full variety reported across the literature when discussing welfare at slaughter.<sup>4,31</sup> To the authors' knowledge, there does not appear to be a previously published structured review of the literature specifically comparing welfare indicators of sheep and/or cattle measured at both stun and non-stun slaughter. The aim of this study was to review the literature comparing welfare indicators of sheep and cattle during stun and non-stun slaughter. The results could benefit a range of stakeholders involved in cattle and sheep slaughter when discussing approaches for new guidance and regulations on animal welfare at slaughter. This research was granted ethical approval by the School of Veterinary Medicine and Science, University of Nottingham, United Kingdom (Project ID: UG20261).

### **METHODS AND MATERIALS**

#### Protocol

This scoping review was reported, where appropriate, according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist.<sup>32</sup> The PICO question for this review was 'In (slaughtered sheep and cattle) does using (stunning vs. non-stunning preslaughter methods) result in (improved animal welfare indicators)?'

## Search strategy

The information sources consulted for study identification were three electronic databases: CAB Abstracts (Ovid) (1910–present), PubMed (1950–present) and MEDLINE (Ovid) (1946–present). The search was conducted on the 12 July 2021. Search terms (Appendix A) were chosen according to the population, intervention and comparator terms included in the PICO question.<sup>33,34</sup> All papers obtained from the three databases were imported into Mendeley, and all duplicates (identified based on title, date published and authors) were removed before screening.

## **Eligibility criteria**

A publication was considered eligible for inclusion if it reported primary research – defined as a study where the author collected data as part of a qualitative, observational or experimental study. The population was required to be sheep and/or cattle of any age or breed. Eligible studies measured at least one indicator of animal welfare during slaughter with and without prior stunning, which were comparable within a single study. References were excluded if the full text was not available through the University of Nottingham library or the British Library, or not published in a peer-reviewed journal.

## **Screening process**

Two stages of screening were carried out. The first stage was completed independently by the first author (Sky Tetlow), and any study with a title that clearly did not fit the eligibility criteria was excluded. In the second stage, the remaining studies were screened by the first author and final author independently (Sky Tetlow and Amelia Garcia-Ara). For this stage, abstracts were read, and the full text was retrieved for any papers where the information contained within the abstract was deemed insufficient for deciding eligibility. Where there was initial disagreement over eligibility, the papers were read and discussed until consensus was reached among two of the authors (Sky Tetlow and Amelia Garcia-Ara). Papers in languages other than English that were not excluded by title/abstract screening were translated using Google Translate to determine whether the article fulfilled the eligibility criteria; these articles were then fully translated if deemed relevant.

## **Data extraction**

Information regarding the study design, species and numbers of animals involved, the restraint method, the method of non-stun slaughter, the measured welfare indicator, the study environment and the authors' conclusions were extracted from the studies that met all of the inclusion criteria.

## Synthesis of results

All data were handled and summarised by one author (Sky Tetlow) and manually tabulated in Microsoft Word (2016) (Table 1).

## RESULTS

### **Study selection**

A total of 1402 papers were initially identified across three electronic databases (CAB Abstracts, PubMed

and MEDLINE; Figure 1). Four hundred and forty duplicates were identified in Mendeley. The remaining 962 titles were screened for eligibility by one author (Sky Tetlow). A second screening of 84 abstracts was completed by two authors (Sky Tetlow and Amelia Garcia-Ara). Two papers were not available through the University of Nottingham or British Library and were excluded.<sup>35,36</sup> Following screening, a total of 16 papers fulfilled the eligibility criteria. One paper was translated from Portuguese to English for inclusion in the study.<sup>26</sup>

## **Study characteristics**

Four studies were conducted in England, 5,19,21,37 three in Italy, 27,28,38 two in Brazil, 26,39 two in South Africa, 24,40 two in New Zealand, 41,42 one in India, 43 one in Mexico<sup>44</sup> and one in France. 29 Three authors were over-represented, each being listed as first authors for two of the selected studies (Table 1). Most environments for the study were commercial abattoirs (Table 1). Halal and shechita slaughter methods were represented separately within equal numbers of studies (n = 5), while two studies measured welfare associated with both methods (Table 1).

The minority of studies (n = 4/16) measured welfare indicators in sheep at slaughter, while the majority of studies (n = 11/16) focused on cattle slaughter. One studied both cattle and sheep.<sup>42</sup>

Half of the studies (n = 8/16) measured one or more biochemical parameters as indicators of welfare at slaughter, in particular plasma cortisol (n = 6), catecholamines (n = 4), glucose (n = 3), lactate (n = 4)and packed cell volume (PCV) (n = 2) (Table 1). One study measured parameters not seen elsewhere in the selected studies, including lactate dehydrogenase (LDH), creatinine kinase (CK) and leukocytes.<sup>26</sup> Of the studies that measured plasma cortisol (n = 6), three concluded that higher cortisol levels were associated with non-stun slaughter,<sup>27,28,40</sup> and none reported the inverse. Of studies measuring catecholamines (n = 4), two concluded that stress was increased at non-stun slaughter<sup>24,40</sup> and two were inconclusive.<sup>26,41</sup> Lactate was measured (n = 4) but the majority of studies (n = 3) made no conclusions based upon lactate concentration at slaughter,<sup>24,26,43</sup> with one study reporting an increased value at non-stun slaughter.<sup>40</sup> One study reported higher leukocyte counts at non-stun slaughter.<sup>26</sup> Some studies measured glucose (n = 3), PCV (n = 2), LDH (n = 1) and CK (n = 1), which were not used to draw conclusions regarding the effects of stunning on stress at slaughter (Table 1).

Electrical brain activity (n = 3/16) and observable behaviours and reflexes (n = 4/16) were measured to determine the extent and point of insensibility after the cut (Table 1). All seven studies measuring consciousness after the cut reported there was increased prevalence of the corneal reflex (n = 3), palpebral reflex (n = 2), righting reflex (n = 1), rhythmic breathing (n = 3), electrical brain activity (n = 3) and evoked visual and somatosensory responses (n = 1)

Reference	Species/number of animals in study NST ST	animals in study ST	Method of restraint NST	ST	Non-stun slaughter method	Parameter(s) measured/time of measurement	Study environment	Authors' conclusion
Bager et al., 1992 <sup>19</sup>	6 Calves	6 Calves	Sedated, sternal recumbency	Sedated, sternal recumbency	Not specified	ECoG Absence of high amplitude low frequency activity = insensible Plasma PO <sub>2</sub> Lactate Haemoglobin During exsanguination	Experimental abattoir	ST = high-amplitude low-frequency activity lost permanently at stun NST = high-amplitude low-frequency ECoG activity lost at 10 seconds after cut = extended sensibility Contradictory blood biochemical parame- ters = Inconclusive
Barrasso et al., 2020 <sup>45</sup>	30 Charolais male cattle	30 Charolais male cattle	FIRP	Upright SB	Halal	PC T1 = 1 week before slaughter T2 = during bleeding	Commercial abattoir	NST = higher PC = increased stress
Barrasso et al., 2020 <sup>28</sup>	120 Lambs	120 Lambs	Limbs only, no head restraint	Limbs only, no head restraint	Halal	Rhythmic breathing and corneal reflex T1, T2 and T3 = 15, 30 and 90 seconds after bleeding, respectively	Commercial abattoir	NST = higher prevalence of corneal reflex and rhythmic breathing = increased extent and duration of consciousness
Bourguet et al., 2011 <sup>29</sup>	95 Adult cattle	95 Adult cattle	FIRP	Upright SB	Halal	Corneal reflex Palpebral reflex At 10 and 30 seconds after cut Rhythmic breathing At 10 and 30 seconds after cut/at stunning pH and temperature of muscle At postmortem	Commercial abattoir	NST = higher prevalence of cranial nerve reflexes and rhythmic breathing = increased extent and duration of consciousness Contradictory postmortem indica- tors = inconclusive (Continues)

Summary of evidence from studies comparing welfare indicators of stunned versus non-stunned cattle and sheep at slaughter (n = 16)TABLE 1

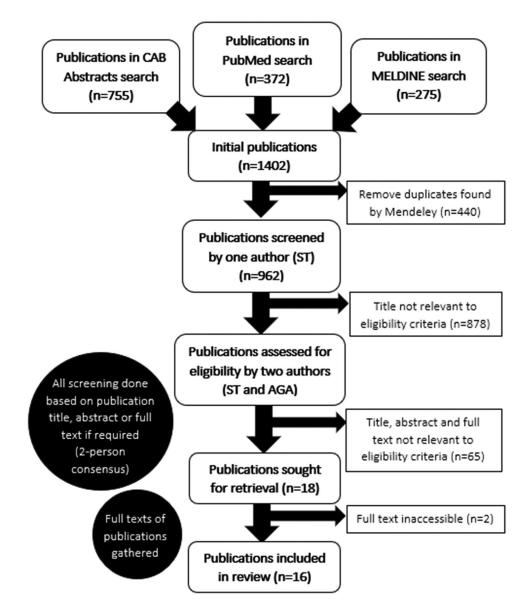
		Authors' conclusion	NST = higher VMA = increased stress	NST = higher PC = increased stress	ST = visual and somatosensory evoked responses lost permanently at stun NST = extended time to reach <10 uV cortical brain activity and <10% visual and somatosensory evoked responses (c.f. stun animals) = increased extent and duration of consciousness	ST = EEG indicative of electroplectic fit and immediate insensibility at stun NST = increased extent and duration of consciousness (Continues)
	Study	nment	Not specified NST VA	Commercial NST abattoir PC	Experimental ST = abattoir St = so abattoir so so new NST received and so so so received and so so received and so	Experimental ST = abattoir ele im
	Parameter(s) measured/time of	measurement	Creatinine corrected urinary concentrations of VMA T1 = basal levels T2 = during exsanguination	PC T1 = during growth T2 = after transport T3 = during exsanguination	ECoG Time taken after cut to reach <10 uV cortical activity and <10% of control values for visual and somatosensory evoked responses	EEG Time taken after cut to reach <10 uV = insensible During exsanguination
	Non-stun slaughter	method	Severing of spinal cord	Shechita	Shechita	Not specified
		$\mathbf{ST}$	Not specified	Upright SB	Upright SB	Sheep: V-shaped box box from the from the floor calves: polypropy- lene net isolated from the floor
	Method of restraint	NST	Not specified	Not specified	Inverted in Weinberg casting pen	Sheep: V-shaped box insulated from the floor Calves: polypropylene net isolated from the floor
	Species/number of animals in study	ST	30 F1 Hol- stein/Zebu cattle	30 Charolais male cattle	8 Cattle	8 Sheep, 5 calves
(Continued)	Species/number of	NST	10 Holstein and 30 F1 Hol- stein/Zebu cattle	30 Charolais male cattle	8 Cattle	10 Sheep, 1 calf
TABLE 1 (		Reference	Caballero et al., 1998 <sup>44</sup>	Ceci et al., 2017 <sup>27</sup>	Daly et al., 1988 <sup>5</sup>	Devine et al., 1986 <sup>42</sup>

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	Species/number of animals in study	f animals in study	Method of restraint		Non-stun	Parameter(s)	Cturder	
Reference	NST	ST	NST	ST	staugner method	measurement	suuy environment	Authors' conclusion
Gregory et al., 2008 <sup>21</sup>	Shechita = 231 cattle Halal = 116 cattle, 11 water buffalo	20 Cattle	Variable	Upright SB	Halal and shechita	Diameter of severed ends of both carotid arteries During exsanguination	Commercial abattoirs	NST = false aneurysms present = increased extent and duration of consciousness
Gregory et al., 2009 <sup>37</sup>	Shechita = 123 cattle Halal = 124 cattle	103 Cattle	Shechita = upright position with a neck yoke, chin lift and belly supporting plate minus belly supporting plate	Upright SB	Halal and shechita	Blood contamination in trachea and bronchi; presence of blood-tinged foam	Commercial abattoirs	NST = irritation from blood in airways and blood-tinged foam indicating post-cut breathing
Kiran et al., 2019 <sup>43</sup>	15 Nellore crossbred male sheep	15 Nellore crossbred male sheep	Lateral manual restraint	Upright SB	Halal	Glucose Lactate During exsanguination	Experimental abattoir	Inconclusive
Neves et al., 2016 <sup>39</sup>	88 Male cattle	279 Male cattle	Shackle and hoist	Upright SB	Shechita	Corneal reflex Palpebral reflex At 20 and 60 seconds after cut Rhythmic breathing At 60 seconds after cut	Commercial abattoir	NST = higher prevalence of cranial nerve reflexes and rhythmic breathing = increased extent and duration of consciousness
Pearson et al., 1977 <sup>41</sup>	A1 = 20 lambs A2 = 20 lambs	A1 = 188 lambs A2 = 46 lambs	Not specified	Not specified	Not specified	PC Norepinephrine Epinephrine During exsanguination	Commercial abattoir	Inconclusive (Continues)

TABLE 1 (Continued)

MeterereNTSTNSTSTNNSTNNStateAutors conclusionPrivetal $A_1+A_2=20$ $A_1+A_2=21$ H1RPUprightSBShechinaPercenterand conclusionand conclusion1991°cattle $A_1+A_2=21$ H1RPUprightSBShechinaPercenterand conclusionand conclusion1991°cattleGost indicasiGost indicasiDisplayPercenterand conclusionand conclusion1994°SolutionesGost indicasiGost indicasiGost indicasiCommercialNST = higher PC inPrivetalGost indicasiGost indicasiGost indicasiGost indicasiCommercialNST = higher PC inStateGost indicasiGost indicasiGost indicasiGost indicasiCommercialNST = higher PC inStateBST = 37 cattleGost indicasiGost indicasiGost indicasiCommercialNST = higher PC inStateBST = 37 cattleIndicasiGost indicasiGost indicasiGost indicasiState in conclusionStateBST = 37 cattleIndicasiGost indicasiGost indicasiCommercialState indicasiStateBST = 37 cattleIndicasiGost indicasiGost indicasiState indicasiGost indicasiStateBST = 37 cattleIndicasiIndicasiGost indicasiState indicasiGost indicasiStateBST = 37 cattleIndicasiIndicasiGost indicasiState indicasiState indicasi </th <th></th> <th>Species/number of animals in study</th> <th>f animals in study</th> <th>Method of restraint</th> <th></th> <th>Non-stun</th> <th>Parameter(s)</th> <th>Churder</th> <th></th>		Species/number of animals in study	f animals in study	Method of restraint		Non-stun	Parameter(s)	Churder	
A1+A2=20 A1+A2=21 HRP Upright SB Shechita PC Commercial NN   cattle attle HIRP Upright SB Shechita PC dattorin   Zebu cattle 60 Zebu cattle HIRP Upright SB Shechita PC commercial NN   Set acttle 60 Zebu cattle HIRP Upright SB Shechita PC commercial NN   Bos indicasi 60 Zebu cattle HIRP Upright SB Shechita PC commercial NN   SNT = 37 cattle indicusi findicusi <td< th=""><th>Reference</th><th>NST</th><th>ST</th><th>NST</th><th>ST</th><th>method</th><th>measurement</th><th>stuuy environment</th><th>Authors' conclusion</th></td<>	Reference	NST	ST	NST	ST	method	measurement	stuuy environment	Authors' conclusion
Zebu cattle 60 Zebu cattle HRP Upright SB Shechta PC Commercial NN   (Bos indicus) (Bos (Bos (Bos (Datal cattle cholamines) (Bos (Datal cattle cholamines) (Bos   NST = 37 cattle indicus) (Bos (Datal cattle cholamines) (Bos	Petty et al., 1991 <sup>40</sup>	A1 + A2 = 20 cattle	A1 + A2 = 21 cattle	HIRP	Upright SB	Shechita	PC Lactate Total catecholamines	Commercial abattoir	NST = higher PC, lactate and cate- cholamines = increased stress
84 Sheep 18 Sheep Lateral Lateral Halal Righting reftex and Commercial ST recumbency on recumbency on recumbency on metal manual limb grid on metal manual limb grid dehydrogenase NN abattoir and head restraint Creatinine kinase PC Lactate PCV Total catecholamines Glucose Leukocytes At 20 seconds after bleeding NN ble	Petty et al., 1994 <sup>24</sup>	Zebu cattle ( <i>Bos indicus</i> ) NST = 13 cattle PST = 37 cattle	60 Zebu cattle (Bos indicus)	HIRP	Upright SB	Shechita	PC Total catecholamines (norepinephrine) and epinephrine) Glucose Lactate PCV During exsanguination, on 3 separate days.	Commercial abattoir	NST = higher catecholamines (c.f. ST animals on same day) = increased stress PST = less stress than NST
	Sterza et al., 2020 <sup>26</sup>	84 Sheep	18 Sheep	Lateral recumbency on metal grid, manual limb and head restraint	Lateral recumbency on metal grid	Halal	Righting reflex and vocalisation Lactate dehydrogenase Creatinine kinase PC Lactate PCV Total catecholamines Glucose Leukocytes At 20 seconds after bleeding	Commercial abattoir	ST = no reflexes immediately after stunning NST = presence of reflex and vocalisa- tion = increased extent and duration of consciousness Similar blood biochemical parameters for ST and NST = increased leukocytes due to

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**FIGURE 1** Results of searches and screening processes used to identify relevant papers (*n* = 16)

in non-stunned animals at slaughter. All indicators of consciousness, except corneal reflex, were absent in stunned animals.<sup>26,28,29,39</sup>

Other studies (n = 3) measured negative welfare indicators not commonly used in the literature (Table 1): the diameter of carotid arteries at postmortem examination (indicator for increased prevalence of false aneurysms and sustained consciousness);<sup>21</sup> vanil-mandelic acid (VMA) urine concentration (indicator of increased stress);<sup>44</sup> the increased prevalence of a blood-tinged foam in the respiratory tract at postmortem (indicator of breathing after the cut).<sup>37</sup>

Across all studies where the method of restraint was specified (n = 12), stunning was performed with the animal in an upright position. In contrast, non-stun slaughter restraint methods differed between studies; upright (n = 2), upright under sedation (n = 2), rotary boxes (n = 5), the shackle and hoist method (n = 1) or manual lateral restraint (n = 2) (Table 1). Four studies specified the use of identical restraint methods in both stun and non-stun animals (Table 1).

#### **Study outcomes**

Most studies (n = 14/16) concluded that slaughter without prior stunning led to increased values for measurable indicators of stress or consciousness (Table 1). The remaining studies were deemed inconclusive ( $n = 2/16^{41,43}$ ), and no papers concluded that stunning before slaughter negatively affected animal welfare indicators at slaughter.

### DISCUSSION

To the authors' knowledge, this is the first scoping review to assess the scientific literature that measures welfare indicators in cattle and sheep after pre-stun slaughter and non-stun slaughter within a single study. This review highlights that most of the literature concludes that better welfare indicators were identified in animals slaughtered following stunning.

The method of restraint affects the concentration of stress indicators in the blood regardless of slaughter procedure, which could have influenced the conclusions reached by studies measuring cortisol and catecholamines. While plasma cortisol concentration is widely recognised as a suitable indicator of stress,<sup>22,46,47</sup> authors debate its reliability as cattle cortisol levels have been shown to increase in natural situations such as mating, parturition, social separation and extended periods of exercise, among others.<sup>23,48–51</sup> Additionally, it is a legal requirement for conventional and religious slaughter practice that animals are individually restrained, which involves separation from the group. This may introduce an additional stress factor in herd species such as sheep and cattle.<sup>3,22,23</sup> Not all studies specified restraint, making it impossible to assess the potential influence of restraint method upon the results of some studies. When reviewing studies that specified non-stun restraint method, it is notable that increased cortisol and catecholamines were only reported during the slaughter of animals restrained in half or full inversion rotary pens. However, studies which used upright or lateral manual restraint methods reported inconclusive results, suggesting that more stressful restraint methods led to higher measurable values of cortisol and catecholamines in the blood. Retained EU regulations stipulate that business operators must ensure that all animals slaughtered without prior stunning are individually restrained and that ruminants shall be mechanically restrained.<sup>2,3</sup> While inversion before slaughter of cattle is illegal under United Kingdom legislation,<sup>2</sup> it is not specifically forbidden under the EU regulations<sup>3</sup> and occurs globally.<sup>52</sup> Hence, due to the variety of restraint methods in daily practice, this review provides valuable insight when forming a comprehensive estimate of animal welfare at non-stun slaughter on a global scale. Based upon the findings of this review, United Kingdom-led research, and collaboration with other countries to further evidence the stress generated through using rotary boxes could discourage their continued use in conscious animals at slaughter.53

False aneurysm formation in the carotids and a slow rate of blood loss may extend the period of consciousness during slaughter without stunning.<sup>38</sup> It is evidenced outside of this review that false aneurysms occur in 17% and 42% of stunned animals cut at the position of the first and third cervical vertebrae (C1 and C3), respectively.<sup>54</sup> Cut position was not specified in the majority of studies in this review, which may lead to variance in studies measuring indicators of consciousness. A legislative change to enforced cutting at the level of C1 to reduce incidence of false aneurysms and the subsequent extended period of sensibility and suffering has been recommended.54-56 However, cut position at slaughter is not specified by any EU or UK law,<sup>57</sup> and halal and shechita slaughter rules only specify that the cut must occur along the windpipe, leaving room for variation in daily practice.<sup>9</sup> Additionally, both studies that controlled cut position to a single location agreed with most previous studies that non-stun slaughter results in extended consciousness. However, further studies where cut

position is controlled are required to validate this finding and ensure comparability of results when measuring welfare indicators at slaughter.

It was noted that studies involving sheep were under-represented among the earlier publications included in this review, while recent studies published after October 2020 could reflect increasing interest in small ruminants at slaughter.<sup>26</sup> This may suggest that the literature is beginning to reflect the large volume of sheep slaughtered without stunning due to halal consumption, in comparison with cattle.<sup>6</sup>

Two studies included in this review each used a welfare indicator not frequently seen in the literature, which may reflect the continuous efforts of the scientific community to establish a definitive measurable indicator for welfare at slaughter. One study measured VMA urine concentration of cattle at slaughter.<sup>44</sup> The author concluded that VMA is a good indicator of acute stress in cattle, and studies have shown urinary VMA to be an indicator for anxiety in people.<sup>58–60</sup> However, the use of urinary VMA concentration remains uncommon in research into animal welfare at slaughter, while a recent study disregarded its ability to indicate anxiety in animals following intensive exercise.<sup>61</sup> An increased prevalence of bloodtinged foam in the respiratory tract at postmortem examination may be indicative of breathing after the cut in the non-stunned animal,<sup>37</sup> which is likely to result in suffering due to irritation and suffocation.<sup>62</sup> Further research is required to determine the usefulness of these less conventional indicators of welfare at slaughter.

Time to onset of insensibility following slaughter can be measured by ECoG and EEG, which involves electrodes on or in the scalp (EEG), or electrodes resting on the dura mater or on the surface of the cerebral cortex (ECoG).<sup>63</sup> Although it has been noted that the indwelling electrodes of ECoG will provide more detailed data that accurately reflect the changing levels of cortical activity, when compared with EEG, both are validated methods used to measure trends in electrical brain activity.<sup>63</sup> Within this review, all studies measuring electrical cortical brain activity indicated that stunned sheep and cattle reach unconsciousness quicker than animals slaughtered without stunning.<sup>5,19,42</sup> Additionally, one study using ECoG reported a wider distribution of results in nonstunned animals (SD =  $\pm 48$  seconds), suggesting a less consistent experience across individuals compared to stun slaughter (SD =  $\pm 1.5$  seconds).<sup>5</sup> All studies reported trends in brain activity at stunning, which suggested immediate and permanent loss of sensibility. 5,19,42

Monitoring procedures in slaughterhouses are based upon behavioural and physical indicators of consciousness in slaughtered animals. According to the European Food Safety Authority, consciousness after neck-cutting should be primarily monitored by loss of breathing and muscle tone, with subsidiary indicators including corneal or palpebral reflexes and vocalisation.<sup>64</sup> Within this review, loss of rhythmic breathing, corneal and palpebral reflexes were assessed in three studies,<sup>28,29,39</sup> while one paper monitored righting reflex and vocalisation.<sup>26</sup> All indicators of consciousness, except corneal reflex, were absent in stunned animals.<sup>26,28,29,39</sup> Corneal reflex is considered the most reliable indicator of consciousness, and hence should not be disregarded.<sup>65,66</sup> While present in 22% of lambs and 3.1% of cattle following stun slaughter, a present corneal reflex was consistently measured in a greater proportion of animals that were not stunned.<sup>28,29,39</sup> The higher prevalence of corneal reflex in stunned lambs (compared with stunned cattle) may be attributed to species differences or stunning method, but further research is required to confirm either trend.

While none of the studies in this review concluded that non-stun slaughter led to improved welfare in comparison with stunning, two papers were inconclusive. It is notable that all papers reporting inconclusive results relied upon blood biochemical parameters of stress markers such as lactate and cortisol. It has been established that biochemical markers of stress are affected by events before the slaughter procedure itself, such as method of restraint and transport, which negate their relevance when directly comparing stun and non-stun slaughter. Few studies were identified during this review that discussed the benefits of non-stun slaughter. While some researchers conclude that a ventral-neck incision is a painless slaughter method rendering the animal instantly insensible.<sup>15–17</sup> studies supporting this conclusion are in the minority across the literature. Additionally, it has been argued that the bleeding of live, non-stun animals is more effective resulting in better meat quality, despite researchers in one study finding no difference in blood loss between stun and non-stun slaughter procedures.67

One study in this review reported that postslaughter stunning also resulted in improved welfare indicators, although more research is required to validate this finding.<sup>24</sup> Other factors surrounding animal welfare at slaughter include transport, lairage and neck-cut position. However, the discussion of these factors is not within the remit of this scoping review as they were not controlled for or specified in many of the studies included in the review; further research into these factors could help to improve overall animal welfare at slaughter.

## Limitations of study approach

The restriction of this review to only sheep and cattle may have excluded relevant articles in similar species such as goats and may have resulted in different conclusions if incorporated. Only papers published in peer-reviewed journals were selected, meaning excluded grey literature may have included information relevant to the conclusion reached following this review. Similarly, further work critically assessing the execution and reporting of these studies may result in altered conclusions, but were not within the scope of this review.

# CONCLUSION

Negative welfare indicators are increased during the slaughter of non-stunned cattle and sheep, in comparison to stunned animals. No studies included in this scoping review concluded that stunning before slaughter resulted in poorer animal welfare when compared with non-stunning. Hence, this review supports the view that stunning is a suitable method for prevention of unnecessary suffering at slaughter. By summarising the existing scientific evidence in a clear, concise and understandable manner, this study may contribute to further discussions regarding the practice of non-stun slaughter for the supply of meat to religious consumers, in the interest of improved animal welfare. The results of the study can be used by animal welfare advisors, politicians, religious communities and others interested in animal welfare to encourage discussions, establish new dialogues when producing new guidance on animal welfare at slaughter.

#### **ACKNOWLEDGMENTS**

This research was supported by the University of Nottingham.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

#### ETHICS STATEMENT

This research was granted ethical approval by the School of Veterinary Medicine and Science, University of Nottingham, United Kingdom (Project ID: UG20261).

#### AUTHOR CONTRIBUTIONS

Study conception and design: Amelia Garcia-Ara, Sky Alexandria Julia Tetlow and Marnie L. Brennan. Literature search and data collection: Sky Alexandria Julia Tetlow. Analysis and interpretation of results: Sky Alexandria Julia Tetlow and Amelia Garcia-Ara. Draft manuscript preparation: Amelia Garcia-Ara, Sky Alexandria Julia Tetlow and Marnie L. Brennan. All authors reviewed the results and approved the final version of the manuscript.

#### DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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# SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Tetlow SAJ, Brennan ML, Garcia-Ara A. Welfare indicators for stunning versus non-stunning slaughter in sheep and cattle: A scoping review. Vet Rec. 2022;e1739. https://doi.org/10.1002/vetr.1739