An independent scientific review of processing establishment practices for livestock welfare.

FEBRUARY 13

Authored by:
Dr Leisha Hewitt and Dr Alison Small

Introduction

This purpose of this review is to provide a critical assessment of published scientific literature in the period 2000-2021, focused on the welfare aspects of livestock (cattle, sheep, goats, pigs, horses, donkeys, camelids, buffalo, rabbits, poultry and other fowl) handling and processing for human consumption in abattoirs.

This review was funded by the Queensland Department of Agriculture and Fisheries (DAFBCP019).

INTRO1: Project objectives

Intro1.1: Project terms of reference

The key deliverable is a written report containing a fully referenced scientific literature review on the welfare of livestock at meat processing facilities. For the purposes of the review, 'Processing facilities' includes abattoirs, knackeries and mobile slaughter services. There is no upper or lower limit on the size of facility to be considered. The literature review is to cover all of the main commercial livestock species that are handled in Australian processing facilities (see search criteria for detail). In regard to poultry, the review is to reference the Farmed Bird Welfare Science Review and identify any new publications since 2017. The literature review is to be based on all relevant and reasonably accessible contemporary scientific research findings, other literature reviews and other published information on the key areas for review (Table 01).

Table 01: Key areas for review as presented in Schedule 1 of the project contract

	Key areas
a	Design and construction of meat processing facilities (including raceways, alleys, walkways and gates) and equipment (handling aids) to minimise the risk of injury to animals.
b	Design and construction of meat processing facilities (including raceways, alleys, walkways and gates) and equipment (handling aids) that facilitate the movement of livestock with minimum handling and reduced stress to the animals.
c	Design of meat processing facilities including holding yards, pens and lairage in the context of Australian climatic conditions (including provision of shelter, food and water during holding periods).
d	Design and use of livestock washing facilities prior to slaughter in the context of Australian climatic conditions.
e	The effectiveness (or otherwise) of closed-circuit television or other methods of remote monitoring of livestock at processing facilities at key animal welfare points in improving welfare outcomes.
f	Factors that may require an adjustment to processing rate to minimise adverse welfare outcomes, such as class, species or breed of animal.
g	Humane handling of foetuses and animals born during transport to / or at a meat processing facility.
h	Management of vulnerable animals for prompt slaughter, including emergency slaughter.
i	Segregation of vulnerable animals to minimise adverse welfare outcomes.
j	The use of dogs to manage livestock at the meat processing facility, considering the welfare of the livestock and the dogs.
k	The use of electric prodders and goads on livestock at meat processing facilities.
l	Design of restraint equipment for slaughter (and treatment or humane killing if necessary) in relation to particular species.
m	Stunning methods and equipment, including: appropriate (or inappropriate) methods of stunning for particular species or classes of animal; welfare benefits and drawbacks of reversible pre-stunning and immediate post-slaughter stunning methods, and methods of stunning not currently covered by Model Code (e.g. LAPS, non-aversive gas stunning).
n	Slaughter without stunning.
o	Appropriate (or inappropriate) methods and techniques for bleeding particular species or classes of animal, including stun to stick intervals.
p	Animal welfare indicators relevant to the operation of processing establishments (including unloading, handling in lairages, stunning and slaughter).

INTRO2: Methodology

Intro2.1: Literature search strategy

This was a systematic review of published scientific literature in the period 2000-2021, focused on the welfare aspects of livestock (cattle, sheep, goats, pigs, horses, donkeys, camelids, buffalo, rabbits, poultry and other fowl) handling and processing for human consumption in abattoirs.
The literature search utilised the CSIRO library database subscriptions. The electronic literature databases included were:

- Web of Science®
- Scopus®
- Agricola®
- Derwent Innovations Index®

The search was completed as an iterative process by species between April and August 2021. Articles identified during the search were uploaded to EndNote reference manager and duplicates automatically detected and removed, followed by manual removal of any additional duplicates (e.g. publications published in more than one format or indexed in more than one database). Articles identified in the search were also exported to an Excel spreadsheet for sorting, alignment and synthesis.

Intro2.2: Article screening and selection

After the initial search and screen, 3003 records were identified (containing 2955 articles and 48 patents). These underwent a further screening process to identify target documents for critical appraisal, through evaluation of each title and abstract.

Intro2.2.1: Eligibility criteria

Inclusions

- Papers relevant to terms of reference detailed previously
- Date of publication: Articles published between 2000 – 2021
- Geographic focus: Worldwide
- Reviews to be included if relevant (e.g. the Farmed Bird Welfare Science Review 2017)

Exclusions

- Papers that focus exclusively on meat quality/safety, with no reference to animal welfare
- Philosophical/opinion papers
- Publications that are not written in English, French or German

Intro2.3: Scope

The scope of the review is the years 2000 - 2021, although the final review contains reference to older papers either if these are seminal works or there has been insignificant work on a particular topic since that time.

Intro2.4: Geographical location

The literature search methodology protects against unintentional bias in selection of papers for inclusion in the review. The papers that have been included come primarily from Europe and North America. In reading the review it should be acknowledged that factors relating to Australian conditions, environment, and established transport and farming practices may not be fully represented in the literature, however, this is discussed by the authors.

Intro2.5: Review protocol

During preparation, the report underwent an iterative review process by the client, and the final report was reviewed via the CSIRO internal review procedure, whereby it was reviewed by two researchers from within the CSIRO Business Unit, but not associated with the report preparation process, and a third-party reviewer invited by the CSIRO approver.

INTRO3: Results

Intro3.1: Overview of the literature included in the review

The amount of peer-reviewed research covering the scope of the review varies by species. For some species, such as cattle, pigs and meat chickens, welfare at the time of processing is well-researched. For other species, there is little published information. Although this review focuses primarily on peer-reviewed articles, additional information from conference proceedings, industry standards and guidance, and information from animal welfare organisations has also been included where appropriate.

Intro3.1.1: Cattle and calf literature

Using Web of Science® core collection, the search string TOPIC: ((abattoir OR slaughter) AND (cattle OR calf OR calves OR bovine) AND (welfare OR handling)) in the period 2000 – 2021 returned 844 articles. Pre-screening of the titles and abstracts according to the exclusion criteria led to 511 articles excluded, and 333 remaining in the database. Of these, 214 were specifically pertaining to cattle or calves in an abattoir processing environment, and the remainder were either of a general nature, applicable to all species, or were specific to slaughter without prior stunning. Within the 214 articles specifically pertaining to cattle or calves, 31 were review articles, 130 were not relevant, being focused on transport, on-farm handling, animal temperament or meat quality. 53 articles were considered to be directly relevant and utilised in the literature review.

Intro3.1.2: Buffalo literature

Using Web of Science® core collection, the search string TOPIC: ((abattoir OR slaughter) AND (buffalo OR bison) AND (welfare OR handling)) in the period 2000 – 2021 returned 29 articles. Pre-screening of the titles and abstracts according to the exclusion criteria led to 23 articles excluded, and 6 remaining in the database. One was a review, one was a technical evaluation of a free bullet device, leaving three that contained relevant material.

Intro3.1.3: Pig literature

Using Web of Science® core collection, the search string TOPIC: (abattoir OR slaughter OR lairage OR handl* OR stunning) AND (pig OR hog OR swine) AND welfare in the period 2000 – 2021 returned 987 articles. Pre-screening of the titles and abstracts according to the exclusion criteria led to 740 articles excluded, and 247 remaining in the database. Of these, 157 were specific to the welfare of pigs in an abattoir processing or farm environments. Within the 157 identified, 46 were not relevant to welfare during, being focused on specific on-farm issues, animal temperament, meat quality and transport. The remaining 111 were considered to be directly relevant and utilised in the literature review.

Intro3.1.4: Sheep and goat literature

Using Web of Science® core collection, the search string TOPIC: ((abattoir OR slaughter) AND (sheep OR goat OR bovine OR caprine OR lamb OR kid) AND (welfare OR handling)) in the period 2000 – 2021 returned 503 articles. Pre-screening of the titles and abstracts according to the exclusion criteria led to 308 articles excluded, and 195 remaining in the database. Of these 23 were relevant to handling and slaughter of goats in commercial abattoirs, and 95 were relevant to sheep. The remaining 77 articles were either of a general nature, applicable to all species, or were specific to slaughter without prior stunning. Within the 118 articles directly pertaining to sheep or goats in a commercial processing environment, 10 were review articles, 75 were not relevant, being focused on transport, on-farm handling, animal temperament or meat quality. 33 articles were considered to be directly relevant and utilised in the literature review.

Intro3.1.5: Horse and donkey literature

In Web of Science, using the search criteria (abattoir or knacker* or slaughter) AND (horse or foal or donkey or equine or equid) AND (welfare or handling), in the period 1900 – 2021, a total of 88 articles were identified. Pre-screening of title and abstract according to the exclusion criteria led to 38 articles being excluded, with 56 remaining in the database. Of these, five were overview/opinion/policy type documents and twelve were review articles or book chapters, discussing issues relating to unwanted horses and transport of horses, horse training and teaching of euthanasia in the veterinary curriculum. Of the remaining 34 research articles, 25 were related to transportation stress and injuries; one related to stress in saleyards, three related to understanding the reasons for horses being unwanted and the limitations of alternatives to slaughter, two investigated stunning methods and six examined physiological or physical markers of stress of injury during the pre-slaughter phase. No article provided scientific evaluation of different facilities or handling methodologies in the pre-slaughter or slaughter phases, although some potentially relevant findings or commentary were identified.

Intro3.1.7: Camelid, deer and guinea pig literature

Using Web of Science® core collection, the search string TOPIC: ((abattoir OR slaughter) AND (camel OR alpaca OR vicuna OR guanaco OR cria OR deer OR antelope OR ‘guinea pig’) AND (welfare OR handling)) in the period 2000 – 2021 returned 50 articles. Of these, 17 were review articles and 20 were not relevant, being relating to process hygiene, animal health or meat quality. The remaining 13 articles were utilised in the literature review.

Intro3.1.8: Rabbit literature

For rabbits, the search terms TOPIC: ((abattoir OR slaughter) AND (rabbit) AND (welfare OR handling)) in the period 2000 – 2021 returned 7 articles. Of these, 6 articles were utilised in the literature review, with the remaining article not being relevant as a newsletter article reporting on one of the other papers.

Intro3.1.9: Meat and layer chicken literature

For meat and layer chickens, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2017-2021. No distinction was made between the type of chicken and the search was limited to articles. The following subject terms were used; chicken AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR "carbon dioxide" OR CO2 OR "controlled atmos" OR "Low atmos" OR laps OR waterbath OR "water-bath" OR "water bath"). The search identified 231 articles, of which 21 were retained in the database after pre-screening of the title and abstract according to the exclusion criteria. Of these 6 were overview/opinion/policy type documents or magazine articles. The remaining 15 research articles covered areas such as stunning systems (waterbath and low atmospheric pressure (LAPs)), lighting, handling, welfare assessment and feed withdrawal. 14 were related to broiler chickens and 1 article related to laying hens.

Intro3.1.11: Turkey literature

For turkeys, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2017-2021. The following subject terms were used; turkey AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR "carbon dioxide" OR CO2 OR "controlled atmos" OR "Low atmos" OR laps OR waterbath OR "water-bath" OR "water bath"). The search identified 103 articles, of which 11 were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.12: Duck literature

For ducks, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; duck AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR "carbon dioxide" OR CO2 OR "controlled atmos" OR "Low atmos" OR laps OR waterbath OR "water-bath" OR "water bath"). The search identified 26 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.13: Goose literature

For geese, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; geese OR goose AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR "carbon dioxide" OR CO2 OR "controlled atmos" OR "Low atmos" OR laps OR waterbath OR "water-bath" OR "water bath"). The search identified 28 articles, of which 1 was retained in the database after pre-screening of the title and abstract according to the exclusion criteria. The identified study from 2020 investigated the effect of high frequency stunning on meat quality in geese, however, it did not measure or record any animal welfare parameters.

Intro3.1.14: Quail literature

For quail, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; quail AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 17 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.15: Ostrich and emu literature

For ostrich and emu, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2017-2021. The following subject terms were used; ostrich* or emu AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 11 articles, of which 1 was retained in the database after pre-screening of the title and abstract according to the exclusion criteria. During the review, 4 additional articles (pre-2017) were identified and added to the database. No articles relevant to the handling and processing of emu were identified.

Intro3.1.16: Guinea fowl literature

For guinea fowl, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; “guinea fowl” AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 9 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.17: Pheasant literature

For pheasant, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; pheasant AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 29 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.18: Partridge literature

For partridge, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; partridge AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 21 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.19: Pigeon literature

For pigeon, the search terms used in the Farmed Bird Welfare Science report (2017) were utilised with the addition of handling, for the period 2011-2021. The following subject terms were used; pigeon OR squab AND ((Stun* NOT stunt) OR kill* OR slaughter* OR cull* OR shackl* OR lairage OR CAS OR CAK OR “carbon dioxide” OR CO2 OR “controlled atmos” OR “Low atmos” OR laps OR waterbath OR “water-bath” OR “water bath”). The search identified 20 articles, of which zero were retained in the database after pre-screening of the title and abstract according to the exclusion criteria.

Intro3.1.20: Non-stunned slaughter literature

Literature pertaining to slaughter without prior stunning (non-stunned or unstunned slaughter) was collected during the course of the larger search activity. 75 papers published in the period 2000 – 2021 were identified as relating to unstunned slaughter. Of these, 22 were review papers, 10 were relating to meat quality with no welfare aspects, 9 were not relevant to Australian processing conditions, 5 were social science surveys of attitudes to unstunned slaughter and 6 were methodological in nature, leaving 23 papers relevant to the literature review.

Intro3.2: Format of the report

The general section of the review covers animal welfare principles and practices that are common for all, or several species. The rest of the review is divided into species sections and focuses on research specific to each animal type. A summary has been produced for those sections that contain a lot of technical information in particular when there may be some conflicting research results.

General

The purpose of this section is to provide an overview of the general principles and processes that apply to all or several livestock species from arrival at the processing establishment through to slaughter. It also examines how pre-slaughter treatment, stunning and slaughter can compromise animal welfare by exposing livestock to conditions that result in pain, fear and distress. This review does not cover further processing once death has been confirmed.

GEN1: Processing establishments and processes

Gen1.1: Processing establishment types

In this review, a processing establishment is a facility where animals are slaughtered for human consumption; often referred to as an abattoir or slaughterhouse. The term poultry processor is used to describe a specific type of establishment where poultry are slaughtered for human consumption. A knackery is an establishment where animals are usually processed as animal feed or by-products. Mobile butchers can be as simple as a single operative killing livestock on the holding of origin (usually using farm facilities); or can comprise of a sophisticated self-contained mobile unit, although this is not currently permitted for commercial slaughter in most states (1), a mobile farm abattoir (Provenir Pty Ltd) was granted a licence in 2019 to operate in NSW. With the exception of livestock killed on-farm in a non-commercial context, RSPCA Australia has completed a review of the regulatory oversight for each of these establishments (2). The RSPCA Australia regulatory review (2) does not cover the slaughter of ratites (ostrich and emu) and rabbits.

Gen1.2: Processes covered by the review

Livestock processing is made up of a sequence of process steps from livestock arrival at the abattoir through to slaughter and the death of the animal, with each part of the process having an impact on the overall animal welfare outcome. The Farm Animal Welfare Council (FAWC) (3) summarised the principles that need to be considered:

“Slaughter [...] is the final event in a farm animal’s life. The following principles must be observed if slaughter […] is to be humane with minimal pain, suffering and distress:

- *All personnel involved with slaughter […] must be trained, competent and caring*
- *Only those animals that are fit should be caught [or penned], loaded and transported to the slaughter site*
- *Any handling of animals prior to slaughter must be done with consideration for the animals’ welfare*
- *In the slaughter facility, only equipment that is fit for the purpose must be used*
- *Prior to slaughter of an animal, either it must be rendered unconscious and insensible to pain instantaneously or unconsciousness must be induced without pain or distress*
- *Animals must not recover consciousness [before] death ensues.”*

A summary of the typical process steps (and those covered by the review), together with the associated welfare considerations is presented in Table 02.

Table 02: Processes covered in the scope of the review

Process	Welfare Consideration
Arrival of stock and unloading	Unloading of livestock at the processing establishment Handling equipment Design, operation and maintenance of facilities for unloading Demonstrated staff competencies
Lairage and holding areas	Access to water Stocking density Shelter Mixing, aggression and animal behaviour Provision of feed and water Design, operation and maintenance of holding facilities Demonstrated staff competencies Animal inspection and emergency slaughter Management of livestock with specific animal welfare needs e.g., pregnant animals, new born animals and their dams, bobby calves
Movement to the slaughter floor	Design, operation and maintenance of handling facilities Handling and drafting of animals Demonstrated staff competencies
Stunning	Design, operation and maintenance of restraint and stunning equipment Restraint and stunning procedure Effective stunning with appropriate equipment Back-up stunning Demonstrated staff competencies
Slaughter	Effective and humane slaughter procedures Maintenance and design of slaughter equipment and facilities Demonstrated staff competencies Slaughter of pregnant animals

Gen1.3: Responsibilities for animal welfare

The principle that supervising and managing animals affects animal welfare is widely recognised within the livestock processing industry. Indeed, the stockperson may be the most influential factor affecting animal handling. Animal handlers require a range of well-developed husbandry skills and knowledge to effectively care for and manage livestock from arrival at the abattoir through to slaughter. It has been realised that training animal handlers to improve human–animal interactions involves modification to their behaviour in addition to knowledge and skills training. Practically, this involves animal handlers learning to behave in different ways by changing the beliefs and attitude that underpin their behaviour and then changing the behaviour itself (4-6).

There is a clear, continuing need for the livestock processing industry to train their personnel to effectively care for and handle their stock, which would be aided by the provision of training in the basic principles of contemporary animal welfare science. There is also an increasing emphasis placed on the competency of stockpeople by the livestock industry and wider community. Many abattoirs have identified specific personnel with responsibilities for overseeing animal welfare. Domestic abattoirs in NSW are required to have a trained Animal Welfare Officer, while establishments processing RSPCA Approved animals are required to have an

Animal Welfare Officer who is competent in all facets of production and who is responsible for the oversight of animal welfare at the facility and for reporting breaches of animal welfare to management to ensure appropriate actions are taken to address breaches (2).

GEN2: Physiological basis of animal welfare at slaughter

Gen2.1: Pain, fear and distress

The broadly accepted description of pain used in animal welfare studies is aligned with the published definition by the International Association for the study of pain (IASP) which states that “An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage” (IASP, 2012, updated from the 1979 definition to include individuals who cannot verbally articulate their experience, for example, animals). The neuropsychological system that regulates the perception of pain in man and animals is the nociceptive system; the function of which is similar in all mammalian species and birds (7). Differences between animals can be found in their reactions to end, to avoid and to cope with pain (8). The expression of pain differs between individuals and between species, with prey species often not showing overt signs (7, 8). During the slaughter process itself pain can be caused by prior injuries, inappropriate handling and restraint, during incorrect stunning and during neck cutting (if stunning is not used or the animal is stunned ineffectively) (7).

Fear is an unpleasant emotional state induced by perception of danger or potential danger, that threatens the integrity of the animal (7). It involves physiological and behavioural changes that prepare the animal to cope with the danger. Fear becomes a problem during the slaughter process when animals encounter novel or unexpected stimuli during holding in lairage, handling and restraint. The features of the animal’s environment, for example, lighting, noise, movement of people and design of the facility may all induce fear (9-12). Many of the reactions of livestock towards humans are attributed to fear. The experience and expression of fear can differ between species and animal type (7). The expression of fear can involve increased agitation and activity in animals. This in itself can create additional animal welfare problems, such as the inability to accurately stun the animal.

The terms stress and distress are often used in a similar context to describe the response to and impact of the conditions that animals are exposed to. Stress is physiological disturbance, which is imposed by a stressor, such as a threatening situation. It involves the activation of the hypothalamic-pituitary-adrenal (HPA)-axis and the activation of the sympathetic nervous system (SNS); common to all vertebrates. The pre-slaughter handling of livestock involves several stages that can cause stress (13-15), including:

- Unloading from the transport vehicle
- Animal handling and movement through the system
- Food withdrawal
- Washing livestock, for example, using hoses
- Restraint for inspection and verification of identification
- Penning and social separation or mixing with unfamiliar animals

The OIE (15) define distress as the state of an animal that has been unable to adapt to stressors, and that manifests as abnormal physiological or behavioural responses. It can be acute or chronic and may result in pathological conditions.

Gen2.2: Consciousness and unconsciousness

There are many definitions of consciousness, but in general it is associated with the awake state and the ability to perceive, interact and communicate with the environment and others (16). Unconsciousness (the opposite to consciousness) is defined as: 'a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence of this disruption, the unconscious animal is unable to respond to normal stimuli, including pain'(17). If an animal is conscious, or if it regains consciousness pain, fear, and distress can be experienced. For slaughter after stunning, this will be relevant where an animal is ineffectively stunned or regains consciousness before death occurs. During slaughter without stunning the animal can be subjected to pain, fear and distress for a period of time until consciousness is lost (7). Determination of the humaneness of methods used to produce unconsciousness in animals, during humane slaughter, relies on an ability to assess stress, pain, and consciousness within the contexts of method and application (18). The brain structures in mammals involved in the generation and maintenance of consciousness in mammals are also present in birds (7). If the respective brain structures do not function, consciousness will be lost. Loss of consciousness can be seen as a process, which, depending on the stunning and slaughter method used, may take some time. Accordingly, the indicators of consciousness and unconsciousness must take into account the species under study and the stunning or slaughter method being used. Animal-based measures that can be used to indicate consciousness and unconsciousness are described in Section 3.1.

GEN3: Animal welfare monitoring

Gen3.1: Animal-based measures

Gen3.1.1: Behavioural measures of unconsciousness and insensibility

To determine whether the stunning and slaughter method is effective, there have to be accurate measures of unconsciousness and insensibility, and persistence of unconsciousness until death. There are various reviews of the indicators of consciousness and unconsciousness within the context of slaughter (7, 18) that cover some of the main livestock species and summarise the pros and cons of each method. The reviews indicate that the most frequently used indicators are those which can be used practically in an abattoir environment. These are often behavioural indicators, such as loss of posture, presence of rhythmic breathing, and vocalisations. An assessment of brain function (for example, presence of spontaneous blinking or corneal reflex) are also useful. However, the authors recommend a cautionary approach when using certain methods for specific stunning processes. For example, the use of eye reflexes as an indicator of effective electrical stunning can be difficult (18). It is also recommended that the indicators are not used in isolation, but in combination with each other. This 'tool-box' approach has been described in detail for the main livestock species in various European Food Safety Authority (EFSA) reports (19-23). EFSA have also published guidance on the assessment criteria for studies evaluating the effectiveness of stunning methods regarding animal protection at the time of killing (17, 24), which focuses on the use of a number of animal-based measures to assess stunning.

The recording and subsequent assessment of brain activity, as presented in an electroencephalogram (EEG), is considered the most objective way to assess unconsciousness compared with reflexes and behavioural indicators, however, this is only feasible in an experimental environment. What is useful from a practical assessment perspective, are the studies which align the results of an EEG assessment with the behavioural indicators which we see in the animal.

Gen3.1.2: Behavioural measures of pain, fear and distress

The movement from the lairage pen to the stunning area constitutes one of the key animal welfare monitoring points in the abattoir. Measures of movement and handling, such as animals slipping or falling and the use of electric goads are useful indicators of the effectiveness of the handling system and process. In addition, occurrences of vocalisation (validated for cattle and pigs), immobilisation, backing-up and turning back are useful indicators of fear and distress during the handling process (25-27).

Vocalisation is a specific behaviour that accompanies particular mental states in livestock. Hence, the presence of vocalisations in some species may supply us with information on their general wellbeing. For this reason, the analysis of livestock vocalisation has gained increasing interest in an attempt to determine the significance as an animal welfare assessment tool. Work on developing modern techniques of sound analysis have provided equipment to discriminate, analyse and classify specific vocalizations (28), with a view to eventually providing tools that can be used as a non-invasive technique for animal welfare assessment in a practical environment.

Gen3.1.3: Physiological stress biomarkers

Stress biomarkers reflect the pathophysiological responses to stress. They can be classified into four groups according to the physiological system or axis evaluated: sympathetic nervous system, hypothalamic-pituitary-adrenal axis, hypothalamic-pituitary-gonadal axis and immune system (29). Ideally, biomarkers should be used in combination to assess and evaluate the stress resulting from diverse causes and the different physiological systems involved in the stress response. Table 03 provides an overview of physiological stress biomarkers that are used in the assessment of animal welfare state. It provides an explanation of the significance of each biomarker in relation to stress in livestock. In addition to the specific stress biomarkers, the table also includes meat quality attributes that have been specifically related to stress in livestock. Other variables include: heart rate, breathing (rate and depth), sweat production, muscle tremor and body temperature (30).

Table 03: Stress biomarkers indicators of livestock welfare

Measure	Examples	Explanation
Stress hormones	Catecholamines (epinephrine/adrenaline, norepinephrine/noradrenaline and dopamine); Cortisol, Adrenocorticotropic hormone (ACTH), Corticotropin releasing factor (CRH)	In a stressful situation, the sympathetic nervous system (SNS) is activated, resulting in the 'flight or fight' response. The **Catecholamines** are released increasing heart rate and respiratory rate, increasing blood flow and oxygen supply to muscles, and increasing the metabolic rate within cells. The catecholamine response is extremely rapid, circulating levels peaking at approximately 30 s after initial stimulus, and then reducing when the stimulus is withdrawn. Simultaneously, the Hypothalamic-pituitary-adrenal (HPA) axis response is triggered. **Corticotropin-releasing factor** is released by the hypothalamus once the stressful stimulus is detected. As a consequence of this, the **adrenocorticotropic hormone** (ACTH) is released by the anterior pituitary gland. Finally, glucocorticoids, e.g., **Cortisol**, are produced and released by the adrenal cortex. Cortisol is one of the most widely used biomarkers to detect stress in livestock. Peak cortisol concentrations occur

		approximately 30 minutes after initial stimulus, and then reducing when the stimulus is withdrawn.
Stress metabolites	Lactate, glucose, free fatty acids, β-hydroxybutyrate, MDA, 4-HNE, 8-OHdG, GSH, GPx, SOD, cholesterol, HDL, LDL, and triglycerides, blood urea nitrogen (BUN)	The glucocorticoids released as a result of the HPA axis response increase the catabolism of tissues rich in glycogen, protein and fat to produce **glucose.** At first, glycogen is metabolised to produce glucose, and under anaerobic conditions (i.e., insufficient oxygen reaching the tissues that are working at a high metabolic rate) lactate as a by-product is released into circulation. When glycogen reserves are depleted, the cells utilise fat and proteins as substrates for energy production, leading to production of **free fatty acids** (FFA), **blood urea nitrogen** (BUN) and **β-hydroxybutyrate**. Alterations in **triglyceride** (cholesterol) levels may also be detected. At this state the body is entering oxidative stress and a variety of lipid peroxidation products such as malondialdehyde (**MDA**), trans-4-hydroxy-2-nonenal (**4-HNE**), 8-hydroxydeoxyguanosine (**8-OHdG**) may be detected in the circulation.
Stress enzymes	Creatine kinase (CK), aspartate amino transferase (AST), superoxide dismutase (SOD), glutathione peroxidase (GPx)	**Creatine kinase (CK)** and **aspartate amino transferase (AST)** are associated with muscle damage and concentrations increase when animals are fatigued. Superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx) are antioxidant enzymes and may be detected in increased concentrations when an animal is under oxidative stress. Their function is to correct the imbalance and return the body to homeostatic balance. Oxidative stress can occur as a result of long-term stress or fatigue.
Acute-phase proteins	haptoglobin, C-reactive protein, albumin, heat shock proteins.	**Acute-phase proteins** (APPs) are blood proteins whose concentration is increased in response to inflammation, infection, and physical or psychological stress.
Electrolytes	Sodium, chloride	Imbalances in **electrolytes** may be associated with dehydration.
Haematology	Packed cell volume (PCV)/haematocrit, Red blood cell (RBC) count, White blood cell (WBC) count and differential; Haemoglobin (Hb)	The majority of haematology parameters are related to animal health and disease as opposed to stress and welfare. However, the **haematocrit,** measured by **packed cell volume** (PCV) can be an indicator of hydration status, A higher PCV would be associated with dehydration.
Meat quality attributes	Muscle glycogen, pH, tenderness (shear force), water holding capacity (drip loss or cooking loss), colour.	As muscle turns to meat after slaughter the **pH** drops on account of **glycogen** being metabolised to lactic acid. When the metabolic rate in increased as a result of acute stress or arousal at the time of slaughter, the **rate of pH decline** increases, leading to a heat toughening situation in which **meat tenderness** and **water holding capacity** can be affected. Meat **colour** can also be affected, appearing lighter than normal. If muscle glycogen is depleted, e.g., through fatigue or longer-term stress, the ultimate pH (pHu) that can be reached is high. This in turn affects meat tenderness, colour and water holding capacity, with the meat industry identifying meat with pH of 5.8 and above as poor quality 'dark cutting'.

Gen 3.2: Monitoring methodology

Industry is being increasingly encouraged to monitor animal welfare as part of their internal management systems and to meet the regulatory requirements and the requirements of external conformity assessment systems, such as the Australian Livestock Processing Industry Animal Welfare Certification System (31) and RSPCA (32, 33).

There has been an increasing shift towards the use of video surveillance in abattoirs. In England, it is a mandatory requirement for business operators of a slaughterhouse to ensure that a CCTV system is installed that provides a complete and clear image of killing and related operations in all areas of the slaughterhouse where live animals are present. RSPCA Australia recommend that slaughtering establishments be required to use CCTV monitoring in all areas where live animals are handled (2), and it is a mandatory requirement in the RSPCA Approved Farming Standards for meat chickens and pigs. A report by Animal Aid, published in the Veterinary Record (34) stated that it is important for the use of video surveillance in abattoirs to be a mandatory requirement in order to be effective. The group also believe that independent monitoring of footage is necessary as the installation of cameras alone was not sufficient to improve animal welfare.

GEN4: Pre-slaughter handling

Gen4.1: Arrival and unloading

Upon arrival at the processing establishment, livestock are unloaded, inspected and placed in the lairage area. During these processes they are exposed to a novel environment that presents different stimuli that can cause fear and stress (6). Unloading is often considered to be one of the most stressful stages of transportation for livestock (35, 36). The stress experienced by the animal at unloading is determined by the characteristics of the species, the individual animal and its previous experiences, the design and maintenance of the facilities and the behaviour of handlers in the area. Within Australia, sheep and cattle, in particular, can be transported long distances in hot temperatures. The unloading of potentially weak and dehydrated animals may present a welfare risk, which may not always be captured in the literature that focuses on European conditions, where transport times are generally much shorter.

Animals with excitable temperaments, for example, those reared extensively with reduced human contact, show more behavioural signs of stress during unloading and handling (10, 37). It is recommended in the literature that unloading commences as soon as livestock arrive at the abattoir (38). Holding animals on a stationary vehicle adversely affects animal welfare and ultimately, final meat quality (39, 40). To reduce the period of time that livestock are held on stationary vehicles, arrivals should be scheduled in a manner that allows for prompt unloading.

Unloading facilities can be improved by the addition of foot battens, rubber mats and deep groove flooring to reduce the risk of livestock slipping and falling. When the design of unloading area is not optimal, using adapted handling methods for unloading, for example, allowing animals to move at their own speed, can improve the process.

Gen4.1.1: Emergency slaughter

Livestock that have been severely injured during transport or are fatigued might only be identified at the time of unloading. The handling and movement of these animals, particularly if they are unable to walk, will exacerbate their pain and distress. In these cases, , emergency slaughter of the animal in situ should be undertaken at the earliest opportunity to prevent further suffering , with the unloading of these animals by any means, including use of a trolley, avoided.(38, 41).

Gen4.1.2: Management of vulnerable livestock

When scheduling livestock for slaughter it is important that the welfare needs of all livestock are taken into account. Examples of vulnerable livestock may include; animals with potentially painful pre-existing conditions, heavily pregnant animals that should not have been transported (42), bobby calves, lactating cows, animals that give birth in the processing plant. In the event that heavily pregnant animals, that should not have been transported, give birth in the establishment, the offspring need to be effectively managed.

Pregnant animals may be sent for slaughter for health, welfare, management and economic reasons; there are also reasons for farmers not knowing that animals sent for slaughter are pregnant. These animals may require a different level of care during the pre-slaughter holding period. It may occasionally be necessary to slaughter pregnant animals at the abattoir. It is important that the impact of slaughter of the dam on the foetus is understood. An EFSA report (43) provides a review of the possible welfare implications of slaughtering pregnant animals, detail of the methods used to kill foetuses and information on how the physical features of livestock foetuses can be used to estimate the dams gestational age. Key conclusions of the review were as follows:

- livestock fetuses in the last third of gestation have the anatomical and neurophysiological structures required to experience negative affect (with 90-100% likelihood).
- It is more probable that the neurophysiological development in foetuses does not allow for conscious perception (with 66-99% likelihood) because of brain inhibitory mechanisms. However, there is also a less probable situation that livestock foetuses can experience negative affect (with 1-33% likelihood) arising from differences in the interpretation of the foetal electroencephalogram, observed responses to external stimuli and the possibility of foetal learning.

Gen4.2: Lairage

The term 'lairaging' is used to describe the holding of animals in stalls, pens, covered areas or fields associated with or part of abattoir operations. It is the period in between the entry of the animals into the facility area (usually unloaded off the truck, but in the case of poultry and rabbits can be on the truck) until moved for slaughter. One of the purposes of the lairage is to maintain an adequate reserve of animals to provide a continuous source of livestock to the slaughter line (44). Another purpose of lairage is to provide an environment which allows stressed or fatigued animals an opportunity to recover (45), however, this outcome is not evident in all livestock species.

The design and operation of the lairage must take consider several provisions and characteristics, such as, space allowance, floor conditions (including bedding), food and water, cooling equipment, lighting, noise and holding duration (15). As with all areas where animals are handled, lairage areas should be free from hazards that may cause injury to animals. Solid floors tend to be preferred to slatted floors, especially if animals are not familiar with slats or perforated flooring. Feeding and drinking equipment should be designed and constructed to allow all animals to have access. There are general provisions for lairage conditions detailed in the OIE Terrestrial Animal Health Code, Chapter 7.5 (15) and in EC 1099/2009 (46), Article 3.2, which states:

"business operators shall, in particular, take the necessary measures to ensure that animals:

> *(a) are provided with physical comfort and protection, in particular by being kept clean in adequate conditions and prevented from falling or slipping;*
> *(b) are protected from injury;*
> *(c) are handled and housed taking into consideration their normal behaviour;*
> *(d) do not show signs of avoidable pain or fear or exhibit abnormal behaviour;*
> *(e) do not suffer from prolonged withdrawal of feed or water;*
> *(f) are prevented from avoidable interaction with other animals that could harm their welfare"*

Gen4.2.1: Thermal and physical comfort

The lairage environment should provide livestock with protection against adverse weather conditions. Heat stress is an important consideration for all livestock species during any holding period in adverse environmental conditions. If the temperature in the lairage is above an animal's thermoneutral zone (checked by measuring temperature and observation of animal-based indicators), mechanisms to cool animals should be provided. Heat mitigation strategies, including showers, misting and fans may also be appropriate for some species. High temperatures and humidity are particularly challenging for pigs and handling should be limited during this time (38). Although not as significant as heat stress, conditions that result in cold stress also need to be considered.

Gen4.2.2: Provision of feed and water

During transport, animals are usually deprived of feed and water, for what may be extended periods. Depending on the time that feed and water were withdrawn and the length of the journey, this may cause prolonged thirst and dehydration. In the lairage, animals (though not poultry) are usually provided with water, though it is common to withhold food unless animals are being held for more than 24 hours (31). The OIE Terrestrial Animal Health Code requires water to be available to the animals on their arrival and at all times to animals in lairages unless they are to be slaughtered without delay (15). Lack of water provision as well as inappropriate design or construction of drinking points that prevent continuous access to water can exacerbate thirst and dehydration. Previous treatment, transport and handling conditions may also influence drinking behaviour of livestock.

Gen4.2.3: Noise and lighting

An abattoir is typically a noisy environment, with sound originating from machinery, handling facilities, animals and sometimes personnel. Loud noises have been shown to increase stress responses in livestock, with intermittent sounds being more disturbing than continuous background noise. The vocalisations of stressed animals and human shouting, were considered to be particularly stressful for livestock (47). Smartphone applications have been developed to monitor noise levels in abattoirs, with results of trials indicating that louder noises occur in the slaughterhall, compared with the lairage and unloading areas (48).

Gen4.2.4: Mixing unfamiliar animals

Mixing unfamiliar animals is to place together animals that were not pen-mates during the rearing period. Usually mixing occurs during selection and loading for transport or less commonly in the lairage pens. Some species and types of livestock may fight after mixing, for example, pigs and young bulls. This type of negative social interaction may cause fear, pain and fatigue. To reduce negative social behaviours and fighting it is recommended to keep animals in familiar groups, particularly in the lairage immediately prior to slaughter (3).

Gen4.3: Animal handling

Poor animal handling is defined as handling where animal handlers frequently engage in negative behaviours such as slaps, hits or prods using electric goads to force livestock to move and include unsuitable facilities, that results in increased stress, fear of humans and poorer meat quality (12).

Gen4.3.1: Handling facilities

Processing facilities appropriate to the effective handling of livestock include raceways, alleys, walkways and gates. When the animals are moving from the lairage area to the stunning point, they generally are

expected to go through a raceway in a single line, with the exception of some group stunning systems for pigs. There is an abundance of scientific evidence to demonstrate that stress during animal movement and the risk of physical injury can be mitigated by ensuring that the animals handling facilities are well designed and operated (12). Many codes of practice and guidelines around the world recommend non-slip flooring, elimination of obstacles and distraction, level surfaces (without ramps and steps) and the provision of good lighting (15, 24, 41). Livestock tend to move better through the facility if visual distractions such as reflections on shiny metal, dangling chains, moving equipment, or people up ahead are removed (49). Sharp contrasts, changes in lighting or light reflections are known to cause animals to baulk during handling (11). It has also been shown that insufficient light intensity
(less than 160–215 lux) at the entrance of the stunning area increases the hesitancy of animals and consequently the use of electric goads (26). Industry is investigating the use of different coloured lights to improve animal movement, with anecdotal reports that green lighting reduces shadows on the floor and improves ease of handling (50).

Gen4.3.2: Handling methods

More recently attention has focused on the animal handling practices in order to optimise animal welfare in processing establishments. Personnel involved in handling and moving of animals should be competent and use species-specific behavioural principles to move livestock (51).Handling equipment includes tools such as electric goads, flags, rattles, boards etc. In Australia, dogs are often used in sheep abattoirs to reduce need for direct intervention between the handler and livestock. Facilities and equipment can influence animal welfare outcomes in two main ways: 1) risk of injury to animals and 2) influence on movement and handling.

Gen4.3.3: Washing livestock

Livestock are sometimes washed at the establishment prior to slaughter. This is usually carried out in the lairage, and the aim is to improve visual cleanliness of the animal, under the belief that this will improve microbial status of the carcase. This belief is in fact unjustified, improvements in carcase microbial contamination being at best negligible, and in some cases worsened by washing of animals prior to slaughter (52-54). Washing of sheep by swimming them through water baths prior to slaughter is utilised in some jurisdictions as a means to reduce the risk of carcase contamination with foodborne microbial pathogens (52). There have been few evaluations of the welfare of sheep subjected to preslaughter swim-washing, but swim washing has a significant effect on meat pHu, indicating that a level of glycogen depletion and stress is induced (55, 56). There is a paucity of data on the welfare of cattle subjected to pre-slaughter washing, but preliminary observations indicate that there is an association between pre-slaughter washing and elevated muscle glucose and lactate (57); or with increased incidence of high pH, dark cutting beef (58). Both attributes are a result of muscle activity and associated with pre-slaughter stress. Furthermore, head-down behaviour (head and ears lowered, indicative of stress or discomfort), has been reported in cattle undergoing pre-slaughter washing, and is associated with increased incidence of dark cutting beef (59).

GEN5: Restraint

The main purpose of restraint is to restrict the movement of an animal; holding it in position for accurate stunning (or slaughter) to be carried out (7, 15). There are several basic principles of restraint that are considered to be important for animal welfare (15). They can be summarised as:

- provision of a non-slippery floor;
- avoidance of excessive pressure applied by restraining equipment that causes struggling or vocalisation in animals;
- equipment engineered to reduce noise of air hissing and clanging metal;
- absence of sharp edges in restraining equipment that would harm animals;
- avoidance of jerking or sudden movement of restraining device.

Restraint is a potentially stressful procedure, hence time in restraint should be kept short. It is also important to manage the entry of the animal into restraint; through effective design and operation of the equipment and the approach (11, 12, 60, 61). The ease of movement of the animal from the holding area into the restraining pen or restraint equipment is likely to vary and is mainly dependant on whether the animals remain in a group or are moved into single file and restrained individually.

The overall welfare of the animal at this point is determined by a combination of factors involving the abattoir environment, the action of the stockman and the response of the animal. Struggling and escape behaviour is often indicative of excessive pressure, whether that be physical pressure applied by the equipment, or pressure applied by the presence of the stockperson in the flight zone of the animal when it cannot move away (61).

The type of restraint method selected depends on the animals to be slaughtered and the process of slaughter (methods, availability of personnel, throughput etc). Typical methods of restraint used for livestock in Australia are summarised in the appendices (APEN1). When slaughter without stunning is used alternative methods of restraint are often employed. This will be discussed further in the relevant species sections.

The restraint of poultry can often involve the use of a shackle line (APEN1); where birds are individually handled and suspended by their legs in a metal shackle, for conveyance to an electrical waterbath stunner. This is covered in section PO1.5.

GEN6: Stunning

Gen6.1: Purpose of stunning

Conscious livestock are capable of feeling pain and distress. The process of stunning livestock before slaughter induces unconsciousness (Section Gen1.2) which avoids exposing animals to any pain associated with the bleeding process. Stunning must induce a state of general unconsciousness until death occurs through the slaughter process (24).

Slight variations exist in the definition of stunning. The Australian *Model Code of Practice for the Welfare of Animals - Livestock at Slaughtering Establishments* (MCoP) (62) describes stunning of animals as '….when it [the animal] is unconscious and insensible to pain. It should not regain consciousness or sensibility before dying'. The World Organisation for Animal Health (OIE) *Terrestrial Animal Health Code* (15) defines stunning as '…any mechanical, electrical, chemical or other procedure which causes immediate loss of consciousness;

when used before slaughter, the loss of consciousness lasts until death from the slaughter process; in the absence of slaughter, the procedure would allow the animal to recover consciousness' and EU *Council Regulation (EC) No 1099/2009 on the protection of animals at the time of killing* defines 'stunning' in Article 2 (f) as 'any intentionally induced process which causes the loss of consciousness and sensibility without pain, including any process resulting in instantaneous death.' The OIE definition infers that stunning is a reversible process, whilst the EU regulation states that stunning may result in the death of the animal.

Fundamental to the concept of stunning is the meaning of 'unconsciousness' (Gen1.2). For stunning interventions that do not induce immediate unconsciousness, any alternative procedure should ensure: 1) the absence of pain, distress and suffering until the onset of unconsciousness, and 2) that the animal remains unconscious and insensible until death. Controlled atmosphere stunning falls into this category as unconsciousness is induced gradually. Under practical conditions, EFSA (24) (17) has defined immediate (or instantaneous) as "unconsciousness occurring within 1 second" of the stun being applied.

Stunning methods are often referred to as 'reversible' and 'irreversible'. Animals will regain consciousness after reversible methods if bleeding (or other killing method) is not performed. With methods regarded as 'irreversible', the majority of animals will not recover from the stun if bleeding is not performed. However, even with irreversible methods, death will often be brought about by blood loss after the bleeding process (7).

Gen6.2: Stunning methods

Stunning methods can be divided into three simple categories: mechanical, electrical and gas (controlled atmosphere). APEN2 in the appendices summarises the typical stunning methods used for the commercial slaughter process in processing establishments. The physiological bases of stunning methods and associated parameters have been thoroughly reviewed, for example by the European Food Safety Authority (EFSA) (24).

Gen6.2.1: Mechanical stunning

Mechanical stunning is a term often used to describe the methods that involve the application of a physical blow to the head of the animal to induce a state of unconsciousness. The theories of concussion have been summarised and presented in a review (63). They are:

- Vascular hypothesis: Where the loss of consciousness is due to a brief episode of cerebral ischemia.
- Reticular hypothesis: Where the loss of consciousness is due to temporary paralyses, disturbances, or depression of the activity of the neural pathways within the brain stem reticular formation and ascending reticular activating system (neuronal activating system).
- Centripetal hypothesis: Where the loss of consciousness occurs due to sudden rotational forces that lead to shearing strains and stresses within the brain; these disengage or disconnect nerve fibres.
- Convulsive hypothesis: In contrary to the vascular hypothesis, the loss of consciousness occurs due to a direct mechanical insult to the neurons leading to initial convulsive phase (tonic-clonic seizures indicative of generalised epilepsy) followed by a quiescent phase.

The author suggests that the neurophysiological data on concussion when mechanical stunning is used is compatible with the convulsive theory. This theory suggests that the energy imparted to the brain by the impact with the animal's head generates movements of the cerebral hemispheres, increasing the chances of an impact between the cortex and the skull, disrupting brain activity and resulting in unconsciousness. Penetrating bolts have additional effects, where the penetration of the bolt induces more widespread brain trauma.

The methods that are used during the processing of livestock that fall into the category of mechanical stunning include:

- Penetrative captive bolt
- Non-penetrative captive bolt
- Firearm (free projectile)
- Blunt force trauma

Although not strictly 'mechanical, the use of neck dislocation to kill poultry is also sometimes placed in this category. This will be considered separately in sections PO1.4 (poultry) and OS3.3 (ostrich and emu).

The category of mechanical stunning method used will be determined by the species and type of animal. In red meat processing establishments, mechanical stunning is normally undertaken using a penetrative or a non-penetrating captive bolt captive bolt device. Penetrative captive bolts are designed to fire a retractable steel bolt through the cranium and into the brain of the animal, whilst non-penetrative devices are not designed to penetrate the skull. Physical signs of an effective stun after the application of a after penetrative or non-penetrative device include immediate collapse, followed by gradual kicking (or clonic) activity (17, 24).

Gen6.2.2: Electrical stunning

Electrical stunning is the passage of electric current through the brain, causing disruption to regular electrical brain activity (64). The applied electrical current causes a depolarisation shift in nerve cells followed by hyperpolarisation of action potentials, which leads to an epileptiform seizure (64-67). The epileptic seizure might cause shorter or longer consciousness loss (66). During the epileptiform seizure, the brain needs a higher oxygen supply than during the normal physiological state, leading to an increase in lactic acid concentration and decrease in pH in the brain. Together with an increase in concentration of neurotransmitters, this causes a loss of consciousness and expression of physical convulsions. In an EFSA scientific opinion it was concluded that electrical stunning is a humane method of rendering an animal immediately unconscious for a duration that will allow death to occur through bleeding (17, 24).

Two types of electrical stunning method are used commercially:

- Electrical head only stunning: involving transcranial application of an electric current
- Electrical head to body stunning: usually involving a head-to-body application of an electric current

Depending on the frequency and amplitude of the electrical current applied, the stun will either be reversible or irreversible (results in ventricular fibrillation) (Section GEN6.1). In red meat animals, the electrical current is either manually applied using hand-held electrodes or is applied automatically via the restraint equipment. Low-voltage electrical stunning, defined as using less than 150 V is not recommended (68) as it is unlikely to deliver the minimum current required to stun the animal. High-voltage systems using 300 V or above are more effective than low-voltage systems and are often used in conjunction with automatic restrainers.

For poultry, the electrical current is usually administered using a waterbath stunner, through which the birds are conveyed using a moving shackle line. This is covered in PO2.1.

Gen6.2.3: Controlled atmosphere stunning

Controlled atmosphere stunning (CAS) methods are used commercially in Australia for pigs and meat chickens. The methodology involves exposing the animals to carbon dioxide (CO_2) or, for chickens, a mixture of CO_2 and an inert gas such as Argon or Nitrogen (under hyperbaric conditions). Stunning is achieved by the increased concentration of carbon dioxide in air (hypercapnic hypoxia), by the depletion of oxygen (anoxia), or a combination of the two (hypercapnic anoxia). The physiological processes that result in unconsciousness involve the regulation of CO_2 and O_2 concentration in the animal's blood (65). When carbon dioxide within a stunning system is inhaled, the majority of the carbon dioxide is transported by blood in HCO_3^- ions. When the buffering capacity of haemoglobin is exceeded, the pH of the cerebrospinal fluid falls as a result of high CO_2 concentration and low blood pH. This induces brain activity depression, leading to loss of consciousness or even to death (64).

GEN7: Slaughter

Gen7.1: Purpose of slaughter

Slaughter is the process of bleeding to induce death, usually by severing major blood vessels supplying oxygenated blood to the brain. After severing the major blood vessels of the neck, animals die due to loss of circulating blood volume and the resultant cerebral anoxia. For an effective stunning and slaughter process, the duration of unconsciousness induced by the stunning period must be longer than the sum of the time taken to perform the neck cut and time required for blood loss to lead to irreversible changes in the brain, resulting in neuronal death (24). The actual process of slaughter is more commonly referred to as 'bleeding' or 'sticking'. These are the terms that will be used throughout the review.

Gen7.2: Slaughter methodology

The method of slaughter, and consequently the blood vessels severed, will be determined primarily by the species of animal. The circulating blood volume in animals is estimated to be 8% of body weight, with specific volumes varying by species (69). During bleeding it has been estimated that animals lose between 40 and 60% of their total blood volume (Warriss and Wilkins, 1987 cited in (7)). Slaughter methods either involve cutting the neck of the animal or performing a thoracic stick (which involves cutting the blood vessels close to the heart). APEN3 in the appendices summarises the typical slaughter methods used for the commercial slaughter process in processing establishments.

Gen7.3: Loss of blood volume and time to brain death

The circulating blood volume in animals is estimated to be 8% of body weight with about 18% of total cardiac output flowing through the brain (24). Cutting the blood vessels leads to a drop in blood pressure and interruption of blood supply to the brain. This results in inadequate oxygenation and loss of consciousness, with the time to loss of consciousness depending on the success of the body's compensatory mechanisms. A literature review presumes that in mammals such as dogs, rats, monkeys and humans consciousness is lost if 30-40% of the total blood volume is lost (70). See also Gen7.4.4: Effect of slaughter technique on bleed-out.

Gen7.4: Slaughter without stunning

Traditional slaughter practice of the Jewish and Muslim religions involves rapidly severing the blood vessels of the neck to initiate a rapid bleed-out. The method is described in the Kosher (Jewish) and Halal (Muslim) food laws, laws which are embedded in the religious texts. In recent years in some parts of the globe, Kosher food processing has allowed post-cut stunning, and Halal food processing has permitted reversible stunning.

Although detail of the specific requirements of each of Kosher and Halal processing differ, three fundamental themes concur: (i) that the animal is whole and undamaged at the time of the neck cut; (ii) that the animal is treated with kindness and respect; (iii) the animal's sacrifice is acknowledged and gratitude is expressed in the correct manner (71-73). The method of slaughter, the rapid cut of the neck, was first documented in the religious laws well over 1000 years ago, long before the stunning technologies used in modern commercial processing were conceived, let alone developed and implemented. With a will to find the balance between themes (i) and (ii) above, a body of research has been published in recent years evaluating the available stunning technologies against these themes, while considering the potential for the animal to make a full recovery from the stun as evidence that theme (i) has been achieved, as compared with traditional unstunned slaughter.

Gen7.4.1: Electroencephalographic studies on pain associated with unstunned slaughter

It has been suggested that as use of an extremely sharp knife during unstunned slaughter can lead to very little behavioural response, the process is not perceived by the animals as painful (71, 74). To investigate this hypothesis, 17 cross-bred calves (109-170 kg liveweight) were studied under very light anaesthesia (75). In 10 calves an arterial bypass system was set up on the neck, so that the blood circulation could be maintained while the tissues of the neck were severed, mimicking unstunned slaughter. In the other 7 calves, the carotid arteries and jugular veins were surgically exteriorised and then severed without damaging the muscles and other tissues of the neck. Electroencephalogram (EEG) recordings were made from the calves to measure neurological responses to the treatments. In the neck-tissue-cutting group, there was an initial increase in F95 spectral edge frequency (i.e., the frequencies detected in the EEG increased overall) and an increase in total power of the EEG, which then dropped and eventually returned to baseline. In comparison, when the blood vessels alone were cut, without damaging the neck tissues, there was a slight decrease in frequency in the EEG and a decrease in total power. The researchers concluded that cutting of the neck tissues resulted in a far greater noxious sensory input to the brain than cutting the blood vessels alone, and that the EEG responses recorded from unstunned slaughter animals are a result of noxious sensory input and not a result of loss of perfusion of the brain. Published concurrently, the same research team characterised the EEG responses of 14 steers (109-162 kg liveweight) to ventral neck incision, compared to a sham procedure (drawing a broom handle across the neck surface) carried out on 10 calves (134-207 kg) (76). Again, the animals were lightly anaesthetised and EEG recordings collected. There was an initial increase in F95 spectral edge in the first 30 s after incision, and this remained stable for 150 s before displaying bursts of periodic activity. Total power at first increased by 60-200%, then fell to around 60% of baseline after 60 s. After 150 s, bursts of periodic activity in total power were also displayed. The sham procedure induced a transient (< 3 s) non-significant increase in total power, and no significant alterations in F95 spectral edge. The authors concluded that ventral neck incision could be perceived as noxious or painful. A further study by this research team investigated the effect of application of a captive bolt stun 5 s after the ventral neck incision on the EEG responses of calves. Utilising the same methodology, 7 calves (134-207 kg) were lightly anaesthetised and subjected to ventral neck incision, followed by non-penetrative captive bolt stunning (instrument not identified) applied 5 s after the ventral neck incision (77). Total power initially increased relative to baseline, but by 30 s after slaughter, was significantly lower than baseline ($P < 0.05$), and by 90 s was around 32% of baseline. Immediately after ventral neck incision, the EEG contained a greater proportion of higher-frequency activity that baseline, and application of the captive bolt resulted first in a period of 'out-of-range' data which resolved to a transitional EEG, High-Amplitude-Low-Frequency or isoelectric EEG, all of which are states that are not compatible with consciousness. The researchers concluded that application of the captive bolt prevented development of the EEG responses that would be considered to be noxious or painful, and resulted in the brain cortex ceasing to function. The experience of the animal in the 5-s period between neck cutting and captive bolt application is unknown, as the EEG entered a transitional high-frequency state.

An Australian study (78) comparing unstunned slaughter (US, with penetrative captive bolt applied after EEG recording) against penetrative captive bolt (P; Cash 8000, 0.22 calibre), low power non-penetrative captive bolt (LPNP; Cash magnum knocker, 0.25 calibre, 3-grain cartridge) and high power non-penetrative captive bolt (HPNP; Cash magnum knocker, 0.25 calibre, 4-grain cartridge) in slaughter-generation steers and heifers (268-635 kg liveweight) provided a randomised design. Median frequency of EEG increased after unstunned slaughter and was significantly different ($P < 0.05$) from median frequency in the stunned groups, all of which decreased significantly compared with pre-slaughter ($P < 0.05$). in the US group, there was also a significant increase ($P < 0.05$) in power in the Alpha and Beta frequency bands (both of which are associated with consciousness), which was not the case in the stunned groups. The authors concluded that the unstunned animals had EEG changes associated with stress and pain and recommended that penetrative stunning would be the most appropriate approach to ensure unconsciousness and minimise pain.

In lightly-anaesthetised goats, a comparison was made of unstunned slaughter against low-frequency head-to-back electrical stunning (LFHB, 1.0 A, 50 Hz, 3 s); low-frequency head-only electrical stunning (LFHO, 1.0 A, 50 Hz, 3 s) and high-frequency head-to-back electrical stunning (HFHB, 1.0 A, 850 Hz, 3 s) using EEG recording (79). All electrically stunned groups showed a significant ($P < 0.0001$) reduction in total EEG power after slaughter as compared with baseline, while the unstunned slaughter group showed an increase in total power. The difference between the unstunned slaughter group and the electrically stunned groups was statistically significant ($P < 0.01$). Median frequency of the EEG also decreased significantly after slaughter in the electrically stunned groups ($P < 0.0001$) but increased significantly in the unstunned group ($P < 0.001$). Again, the difference between the unstunned group and the electrically stunned groups was statistically significant ($P < 0.0001$). The authors concluded that electrical stunning may render the animals unconscious at the point of slaughter.

Gen7.4.2: Effect of slaughter technique on stress-related biomarkers

Plasma cortisol is commonly used to evaluate stress situations in livestock. The cortisol response is relatively rapid, with peak plasma concentrations occurring between 15-30 min after a stressful or painful event. An Italian study (80) compared 8-month-old beef calves that were either slaughtered unstunned in a fully inverted rotary box (n = 30) or slaughtered using captive bolt. Both groups were exsanguinated using a transverse neck cut and blood samples were collected from the free-flowing exsanguinate approximately 30 min later. A baseline blood sample had been collected at the farm one week prior to slaughter, and the calves had been lairaged for 30-45 min after a 45 min transportation. Plasma cortisol at exsanguination was significantly higher in the unstunned group than the captive bolt group ($P < 0.001$), and the difference in cortisol concentration between baseline and exsanguination was significantly greater ($P < 0.05$) in the unstunned (27.5-fold increase) than the captive bolt group (12.85-fold increase). A similar study from the same research institute, reported in two separate papers (81, 82), that a number of stress-related biomarkers (cortisol, dopamine, adrenaline and noradrenaline) were significantly different in the post slaughter exsanguinate in unstunned than captive-bolt stunned 8-month-old beef calves (n = 30 in each group). The results are all the more interesting because the calves selected for unstunned slaughter were selected on farm by Rabbis prior to transportation. They were more docile than those in the captive bolt group, and the plasma cortisol concentrations both on farm and post-transportation were lower (not statistically significant on farm, but significant post transportation; $P < 0.001$) than those of the captive bolt group. Therefore, we could hypothesise that cortisol levels post slaughter would remain lower in this carefully selected group than in the less docile group. This was not the case: at exsanguination, cortisol concentration was significantly greater in unstunned than captive bolt ($P < 0.001$), dopamine was significantly greater in unstunned than captive bolt ($P < 0.05$), noradrenaline was significantly greater in unstunned than captive bolt ($P < 0.05$), and adrenaline was greater (not statistically significant) in unstunned than captive bolt. In the Australian study described earlier (78) (which compared unstunned versus penetrative captive bolt, low-power non-penetrative captive bolt and high-power non-penetrative captive bolt) there were few changes in plasma biomarkers between the immediate pre-slaughter sample and post-slaughter. Penetrative captive bolt elicited an increase in ACTH between stun and slaughter ($P < 0.05$), while high-power non-penetrative stunning elicited an increase in noradrenaline between pre-stun and post stun ($P < 0.05$), but after slaughter was again not significantly different from baseline. There were no other significant changes, or differences between slaughter procedures on cortisol, adrenaline and beta-endorphin.

A Malaysian study (79) comparing unstunned slaughter against low-frequency head-to-back electrical stunning (LFHB, 1.0 A, 50 Hz, 3 s); low-frequency head-only electrical stunning (LFHO, 1.0 A, 50 Hz, 3 s) and high-frequency head-to-back electrical stunning (HFHB, 1.0 A, 850 Hz, 3 s) in lightly-anaesthetised goats found few differences in blood biomarkers between slaughter techniques. The increase in plasma glucose and plasma adrenaline between pre-slaughter and the exsanguinating blood was less in the unstunned groups than the electrically stunned groups ($P < 0.05$ and $P < 0.0001$ respectively). They attributed this finding to the epileptiform state induced by the electrical stun.

Proponents of unstunned slaughter have argued that use of an extremely sharp knife is a key component to ensuring that the animal does not feel pain (71, 74). A Malaysian study evaluated the effect of knife sharpness on plasma stress-related biomarkers and EEG parameters in cattle undergoing unstunned slaughter in a lateral restraint (83). The knives used were 12-inch Victorinox Pro cimeter brand, tested for sharpness using the ANAGO® 300e testing unit. Knives were categorised as 'sharp' (ANAGO score > 8.0) or 'commercial' (mean score 7.8). 10 animals were slaughtered using each category of knife, the cuts being performed at the level of the first cervical vertebra (C1). Blood samples were collected immediately before slaughter, and from the free-flowing exsanguinate. The data are challenging to interpret, as there were differences in means at the pre-slaughter sample, and the statistical analysis does not seem to have accounted for non-independent sampling (e.g., use of a repeated-measures model). However, plasma glucose and creatine kinase were significantly increased from pre to post slaughter in the 'commercial' group ($P < 0.05$) but not in the 'sharp' group, while plasma lactate was significantly increased ($P < 0.05$) in the 'sharp' group but not in the 'commercial' group. Plasma lactate dehydrogenase was significantly increased in both groups ($P < 0.05$). Plasma noradrenaline was unaffected, but plasma adrenaline was significantly increased in both groups ($P < 0.0001$), and there was a significant ($P < 0.0001$) interaction between timepoint and group, meaning that the increase in plasma adrenaline in the 'commercial' group was significantly greater than the increase in the 'sharp' group. The EEG recordings also suggested that there was a difference between the 'sharp' and 'commercial' groups, increases in median frequency, alpha, beta and theta wave power being significantly greater in the 'commercial' group than in the 'sharp' group ($P < 0.005$). The researchers concluded that although all animals experienced physiological stress as a result of unstunned slaughter, use of an extremely sharp knife could reduce the intensity of the stress.

Gen7.4.3: Effect of slaughter technique on behavioural indicators of loss of consciousness

An identified issue leading to sustained consciousness during unstunned slaughter is the development of 'false aneurysms' or 'carotid ballooning', a condition in which the severed arteries of the neck become occluded as a result of the layered sheath of the artery becoming filled with clotted blood and thereby restricting exsanguination. When blood flow through the carotid arteries is restricted, blood can be diverted through the vertebral arterial system into the basi-occipital plexus, maintaining perfusion of the brain. A study of 174 cattle that were slaughtered without prior stunning in an upright restraint in a Belgian abattoir found that 14% regained an upright stance after an initial collapse, and that the average time to final collapse was 20 s (84). there was a long tail on the data with 8% of cattle having a time to final collapse of greater than 60 s. False aneurysms were present in 7% of cattle, and a prolonged latency to collapse was associated with the presence of false aneurysms. Of the 7 cattle that failed to collapse within 75 s, 5 had large false aneurysms, and cattle with no false aneurysms all collapsed within 34 s of the neck cut. In another Belgian study (85), researchers monitored time to collapse (as an indicator of loss of consciousness) in 644 cattle slaughtered in upright restraint without stunning. Cattle slaughtered with a low neck cut (> 2 tracheal rings from the larynx) took longer to collapse (P < 0.01; 18.9 s vs 13.5 s) than cattle slaughtered with a high neck cut (≤ 2 tracheal rings below the larynx), took longer to reach a state where there were no eye reflexes recorded (P < 0.05; 117.9 s vs 99.2 s) and had a greater mean false aneurysm score (a scale of 0 – 5 based on outer diameter of the artery; P < 0.01; 0.8 vs 0.6). There was a positive correlation with false aneurysm score and time to collapse: animals that had larger false aneurysms took longer than 20 s to collapse. The researchers recommended the high neck cut procedure to reduce the mean time to collapse.

The finding that the higher neck cut is associated with reduced formation of false aneurysms has been supported by a number of other studies, using a variety of restraint methodologies. An Italian team of researchers monitored the formation of false aneurysms in 1200 beef cattle (1-2 year-old) that were slaughtered unstunned in a full-inversion rotary pen (86). They correlated the presence of false aneurysms to cut position on the neck, duration of bleed-out and duration of consciousness (measured using rhythmic breathing and eye reflexes). The incidence of false aneurysm formation was greater when the cut was performed at the level of the 3rd-4th cervical vertebrae (10.25%), than when performed at the level of the 1st-2nd cervical vertebrae (7.25%). The presence of false aneurysms accounted for 37.5% of 136 cases of prolonged consciousness (rhythmic breathing or eye reflexes persisting after 90 seconds of exsanguination). The remaining 62.5% of cases of prolonged consciousness were not associated with the presence of false aneurysms, suggesting that reduction in the incidence of false aneurysm formation can reduce but not eliminate the incidence of cases of prolonged consciousness in unstunned slaughtered cattle. In a survey of unstunned slaughter in 13 abattoirs around the globe (Indonesia, China, France, UK) (87), 29% of cattle evaluated showed restricted blood flow and development of false aneurysms. The observations indicated that use of a lower neck cut position was associated with the development of false aneurysms, and the researchers confirmed this observation in a controlled trial of neck cut position in cattle that were stunned using a captive bolt. In the controlled trial (87), 50 cattle were exanguinated using a high neck cut, level with the first cervical vertebra (C1), and 50 were exsanguinated using a low neck cut, level with the third cervical vertebra (C3). In the C3 group, 44% of animals showed early arrest of blood flow (flow stopped within 45 s), compared with 12% in the C1 group (P < 0.001), and mean aneurysm score was 1.34 in the C3 group compared with 0.9 in the C1 group (P < 0.001).

As indicated in that study, false aneurysms are not specific to unstunned slaughter, but can also occur in animals that have been stunned prior to slaughter. In a survey of 387 cattle that had been slaughtered using captive bolt (instrument not detailed) followed by neck cutting, 67.6% had some degree of false aneurysm development and 16% had large false aneurysms (88). A further study compared the prevalence of false aneurysms in 367 cattle and 11 buffalo slaughtered in six abattoirs (89). 20 cattle in the study were slaughtered using electrical stunning with cardiac arrest (2.1 A head-only, followed by 1.4 A nose-brisket; Banss

GmbH system), the others were slaughtered without prior stunning under a variety of restraint methods. False aneurysms were present in between 27 and 46% of unstunned animals, and large false aneurysms in 18 to 36%. No false aneurysms were present in the animals slaughtered after electrical stunning with cardiac arrest. A study in Brazil assessed indicators of unconsciousness (absence of rhythmic breathing, absence of spontaneous blinking, absence of eye reflexes and absence of response to pinch) in 434 *Bos indicus* bulls (90). Within this study, 279 were stunned using a pneumatically-powered penetrative captive bolt (Jarvis brand, 180-190 psi) followed by thoracic sticking; 67 were stunned using a non-penetrative pneumatically-powered captive bolt (Jarvis brand, 160-170 psi) followed by neck cutting and 88 were slaughtered by neck cutting without prior stunning. The stunned animals were restrained in an upright position with neck yoke and chin lift, while the unstunned animals were shackled and hoisted for neck cutting. The study found that 46% of the animals stunned using the non-penetrative instrument required repeat application, compared to only 2% of animals stunned using the penetrative instrument ($P < 0.001$). There were no significant differences between stun methods in the presence of indicators of ineffective stunning (< 1% of animals) at 20 s and 60 s post exsanguination, but animals slaughtered without prior stunning displayed palpebral reflexes (100% at 20 s and 93% at 60 s); corneal reflexes (98% and 60%); rhythmic breathing (100% and 77%); tense jaw muscles (83% and 13%); response to nostril stimulation (7% and 3%); response to tongue pinch (4% at 20 s) and spontaneous blinking (10% at 20 s). The researchers concluded that unstunned slaughter presented a risk that the animals may suffer pain or distress.

Gen7.4.4: Effect of slaughter technique on bleed-out

A strong blood flow is a key indicator of a healthy animal at slaughter, and under traditional unstunned slaughter is used as a means of assessing the suitability of the carcase for human consumption. A study in South Africa (91) compared percentage blood loss (liveweight – weight after bleeding and hoisting) in 170 beef carcases slaughtered by the Kosher method with post-cut stunning (.22 calibre penetrative captive bolt applied 20 s after neck cutting) in an upright ASPCA restraint box with that of 141 beef carcases stunned using a pneumatic captive bolt gun (details not provided). All carcases were allowed to bleed for 8 minutes prior to post-bleeding weight measurement. There were no significant differences in percentage bleed-out between the two groups. A similar study in Turkey (92) assessed the bleed-out of 13 cattle slaughtered by Halal neck cut after hoisting by one leg against 13 slaughtered using a Cash Special penetrative captive bolt instrument followed by hoisting and bleeding using the transverse neck cut technique. The animals were weighed live, and then 2 min after sticking, and blood was collected in a tub placed on a digital balance below the exsanguinating carcase. Collected blood weight was recorded every 10 s. There were no significant differences between the two groups in terms of percentage blood loss, total blood loss and rate of blood loss calculated from these measurements.

In sheep, a study in Turkey (93) compared captive bolt (Cash Special penetrative, n = 12) followed by neck cutting; head-only electrical stunning (350 V for 3 s, n = 18) and unstunned slaughter (n = 30), measuring rate of blood loss by recording the weight of blood drained into a tub placed on digital balance every 10 s, total blood loss and blood loss as a percentage of liveweight. There were no significant differences between stunning method for any of these parameters. A separate study compared unstunned slaughter, head-only electrical stunning (1.0A, 200 Hz, 3 s), and post-cut head-only electrical stunning (1.0A, 200 Hz, 3 s) in lambs (94). In this study, 80 lambs were used for a detailed collection of blood lost in the first 5 s after neck cut, and 360 lambs were evaluated in a commercial processing situation. Liveweight, carcase weight and offal weights were recorded for all lambs and used to calculate percentage blood loss. In the controlled experiment, blood loss at 60 s was significantly lower (P < 0.001) in the unstunned group than in the electrically stunned group, but after 120 s had elapsed, there were no significant differences between groups. Conversely, in the commercial situation, the percentage blood loss was significantly lower (P < 0.05) at 4 min of exsanguination in the post-cut stunned group than the unstunned group (with the pre-slaughter stunned group intermediate and not significantly different from either). The authors interpreted this outcome cautiously, suggesting that the delay between liveweight measurement the day prior to slaughter and processing could have led to changes in gut fill and hydration that in turn could have confounded the calculations. They concluded that there were no significant differences in final blood loss between the different slaughter processes.

A Korean study compared unstunned slaughter (n = 10) and head-only electrical stunning (1 A, 50 Hz, 3 s, n = 10) in Koran back goats, measuring blood loss as a percentage of liveweight (95). There were no significant differences between the two slaughter techniques.

Gen7.4.5: Other welfare considerations associated with unstunned slaughter

If animals are not rendered unconscious prior to slaughter, there is the risk that they may be subject to respiratory irritation from inhaled blood. A study of 247 cattle (96) that were slaughtered without prior stunning in upright restraint, and 106 cattle slaughtered after captive bolt stunning, at three abattoirs in Europe identified that bright red foam was present in the trachea of 36 – 69% of unstunned cattle, and on 0% of stunned cattle (P < 0.05) – the foaming effect is a result of blood being inhaled, mixed and re-exhaled as a result of breathing movements. The presence of frank blood (not foamed) was related more to abattoir than to slaughter method. In one abattoir there were no significant differences between slaughter method in terms of blood in the trachea or blood in the bronchi. However, the other two abattoirs (in which only unstunned animals were assessed), the prevalence of blood in the trachea or bronchi was greater (P < 0.05) than in the abattoir in which both unstunned and stunned cattle were assessed. The researchers concluded that blood enters the trachea and bronchi irrespective of slaughter method, and irritation of the airways is a potential welfare concern in unstunned animals.

Restraint of animals for slaughter without prior stunning can be very variable. In some jurisdictions, complete inversion of animals prior to the neck cut is still permitted, in others a lateral restraint is used and others utilise an upright restraint. The Australian Model Code of Practice (SCARM 79) (62) does not recommend the use of rotating boxes, and although it is not explicitly stated, the inference is that this recommendation refers to conscious cattle. Rotating boxes are widely used in Australia for stunned cattle. Researchers in the Netherlands measured EEG (brain) and ECG (heart) activity in 31 veal calves that were handled using a rotating box, and either slaughtered without prior stunning, electrically stunned (300 V, 50 Hz, 3 s) prior to neck cutting, or post-cut stunned using a captive bolt instrument (Cash Bulldozer 0.25 calibre) (97). Calves were rotated 90°, 120° or 180° prior to slaughter. Heart rate increased significantly (P < 0.05) during rotation, while heart rate variability decreased significantly (P < 0.05), both parameters indicating increased stress in the animals. The EEG was not affected by rotation, but neck cutting led to a slow decrease in signal complexity and in% power in the Beta wave frequency band; both findings indicating a decline into unconsciousness. Corneal reflexes were present until 78 – 194 s after neck cutting. Bothe electrical stunning and post cut stunning resulted in an immediate decrease in signal complexity and % power in the Beta wave frequency. The researchers concluded that rotation of the conscious animal should be avoided as it compromised calf welfare; and pre-cut electrical stunning or post-cut captive bolt stunning should be used to induce immediate unconsciousness.

Section summary: Slaughter without stunning

It is clear from published literature that the action of severing the tissues of the neck is noxious and likely to be perceived as painful by a conscious animal. Furthermore, problems with restricted exsanguination as a result of false aneurysms and other factors as yet unidentified can lead to sustained consciousness and thus an increase in duration of the adverse welfare associated with the pain inflicted. Stunning prior to slaughter does not interfere with blood flow, and results in the animal being unconscious at the time of slaughter. The challenge with many of the existing stunning methods is that the animal is no longer ‘whole and undamaged’ at the time of slaughter (e.g., mechanical stunning resulting in skull and brain damage; head-to back electrical stunning resulting in cardiac arrest).

Cattle and calves

CA1: Pre-slaughter handling

CA1.1: Unloading process

The loading and unloading process for 105 Friesian-Holstein calves (age not reported, mean carcass weight 141 kg) was studied between farm and abattoir in Italy (98). On farm, the calves were individually housed, but were grouped into groups of 15 for transport and lairage. Calves slipped, fell, backed up and baulked most frequently during loading and unloading, with more falls and baulks and greater use of the electric goad during loading than unloading. Where unloading was rushed, or conversely took a prolonged period (probably due to difficulties in moving the calves), carcase bruising was greater. The incidence of severe bruising was associated with rushed handling and a short resting time of less than 10 minutes, but the number of mild bruises (associated with agonistic interactions between the calves) increased when the resting period was greater than 30 minutes. The authors concluded that bruising could be reduced by improving handler training and design of facilities (e.g., use of non-slip flooring).

CA1.2: Lairage facilities

In the USA, it was identified that finished cattle are 12% heavier than in 2000, so a reassessment of space allowance in lairage pens was justified. Using video footage of cattle behaviour overnight, a group of researchers re-calculated the recommended space allowances for animals of different liveweight ranges (99). In order to permit all animals to have sufficient space to lie down simultaneously, they recommended adding 0.093 m^2 per 45.36 kg additional liveweight over a baseline of 1.86 m^2 for a 544.31 kg animal. I.e. Where the mean liveweight of animal is 589.67 kg they should be allocated 1.95 m^2 each; 635.03 kg should be allocated 2.04 m^2; 680.39 kg should be allocated 2.14 m^2 and 725.75 kg should be allocated 2.23 m^2.

Alterations or contrast in lightness or brightness of the facilities has long been known to result in baulking. A study using dairy cows attempted to quantify the relationship between reflected glare from surfaces and the rate of baulking (100). There was a significant effect of reflected glare from a footbath – when no artificial lighting was present, the fewest animals looked at or stopped before crossing a footbath. The number increased as increasing intensities of lightbulb were installed, and the measured reflected glare from the footbath and the surrounding floor surface also increased significantly. When animals baulked, the luminance of the reflected glare from the footbath was approximately 30% greater than when animals did not baulk. The researchers also characterised luminance levels in 11 abattoirs in the UK, finding that in the luminance of reflected glare from wet floor areas was three times (300%) greater than from dry areas, and the glare from shiny metal surfaces was 10 times (1000%) greater than adjacent areas. Although baulking rates were not assessed in the abattoir survey, it is reasonable to assume that baulking rates could be reduced by reducing the contrast between 'bright' and 'dark' surfaces. A study in the USA observed 74 cattle (*Bos taurus,* approximately 24 months-old) unloading and being handled through the lairage to stunning (101). The researchers recorded baulking (stopping, lowering the head or backing up) aligned with the presence or absence of a noisy diesel truck next to the lairage, floor conditions (wet, dry or muddy) and the shadow contrasts on the floor (sharp, soft or none). Sharp contrasts were bright patches of sunlight with clearly defined edges, while soft contrasts were diffuse alterations in floor colour with no bright patches. Cattle were more likely to baulk during unloading when there were sharp contrasts than when there were either soft or no contrasts ($P < 0.001$), and the presence of the noisy truck increased baulking ($P < 0.001$). In this study, 27% of animals slipped when the floor was wet or muddy; the proportion slipping when the floor was dry was not reported, but the inference is that it was zero. The authors concluded that sharp contrasts and unfamiliar noise should be reduced in the lairage environment.

CA1.3: Holding duration

A study that followed 5-6 month-old calves from farm to slaughter, including a 3-hr transport phase and 1.5 hr of lairage identified that the lairage period was sufficient to allow the calves to recover from the acute stress of transport, in terms of plasma cortisol concentrations (102).

In a preliminary paper on 20 Bali cattle, a period of 24 h rest following an 8 h transport period resulted in significant reductions in plasma glucose, cholesterol and uric acid concentrations compared to samples taken immediately on arrival at the abattoir (103). Similarly, a study of 100 cattle in South Africa (104) indicated that lairage duration may have an effect on plasma concentrations of cortisol, glucose or heat shock proteins – however the data were confounded by transport duration, each lairage duration being specific to a particular journey duration, so it is unclear which of transport or lairage had the greatest effect.

A study in Brazil (105) followed 30 Hereford steers from farm to slaughter, assigning them into two equal groups of 3 h or 12 h lairage. The steers had been transported in two loads for 3.5 h. The research focused on meat quality parameters (pH, meat colour, tenderness and muscle glycogen), and found no significant differences apart from in muscle glycogen, which was lower in the 3 h lairage group than in the 12 h lairage group ($P < 0.005$). This suggests that the longer lairage duration allowed a degree of recovery from the physical activity associated with transportation. Two similar studies in Uruguay (106, 107) studied Hereford and Braford (*Bos indicus* cross) steers that had been divided into two different finishing diet groups as well as subdivided into either 3 h or 15 h lairage after a 3.5 h transportation. There was no effect of diet on carcass bruising or pHu, but there was an effect of lairage duration on meat pHu and plasma parameters. The 15 h lairage group had lower pHu ($P < 0.05$), and in the earlier study the 15 h lairage group also had more tender meat ($P < 0.05$) suggesting that the animals had had greater opportunity to recover from the physical activity involved in loading, transportation and unloading than did the 3 h lairage group.

Researchers in Colombia assessed cattle carcases (predominantly *Bos indicus*) at an abattoir for bruising (108). Using a multivariate analysis, fitting transport duration, distance transported, stocking density on the truck, lairage time, carcase weight and cattle class, they identified that bruising was increased when animals were tightly packed on the truck, and when animals were lairaged for a longer period. The prevalence of bruising was 2.1 times greater in the 18-24 h lairage period, compared to 12-18 h ($P < 0.01$). Although consignments were not mixed at the abattoir, some were sourced from saleyards. Saleyard origin was not identified as a significant contributory factor in the analysis, but it is possible that ongoing social reorganisation within the lairage pens contributed to the finding of increased bruising associated with longer lairage periods.

With the aim of setting recommendations for lairage duration after a long transport period, researchers in Italy took blood samples from 39 Limousine bulls that had been transported from Spain, a distance of 2550 km over approximately 54 h (109). The bulls arrived on 5 consignments, and each consignment was assigned either to 'short' (24-36 h) or 'long' (57-59 h) lairage dependent on when they arrived at the abattoir. Blood samples were collected on arrival and from exsanguination after captive bolt stunning. Reductions in plasma cortisol concentration were greater in the long lairage group, suggesting that the animals were better recovered than the short lairage group. CK levels increased during lairage, which may indicate activity in the pens leading to muscle stress, and pHu was higher in the long lairage group than in the short lairage group, also suggesting a greater level of muscle fatigue. A variety of other parameters were measured, with no significant differences in lairage groups, leading the authors to conclude that extending lairage for longer than 36 h did not provide any additional benefit.

Section summary - Holding period

Although data are missing to recommend optimal lairage durations dependent on duration of transport, in general it appears that for cattle that have functioning rumens, lairage periods greater than 3 h are beneficial in terms of recovery, but lairage periods greater than 36 h afford no additional benefit. Conditions during the holding period will strongly influence the ability of animals to rest, for example noise or activity in the area. General conditions, for example to ensure thermal comfort, are discussed in Section Gen4.2.1. Cattle are able to tolerate low temperatures, however, calves are adversely affected by cold environmental temperature, particularly during transport and holding in the lairage prior to slaughter (38).

CA1.4: Handling techniques

A study in the USA looked at the potential benefits for the management of heat stress of showering *Bos taurus* cattle once prior to handling in a feedlot setting (110). The ambient temperatures recorded during the trial ranged from 22 – 32 °C. 64 heifers were involved in the experiment, half of whom were soaked with water for 20-30 s, on a single occasion while waiting in the race prior to handling (weighed, then held in the handling crush for 20-30 s). The wetted animals had a lower peak rectal temperature and lower panting score than the animals that were not wetted. The author concluded that although a single wetting event did not completely offset heat stress, it was a beneficial treatment. This finding is useful when considering the constraints on water supply in Australian processing. A further study in the USA investigated the degree of aversion to increasing flow rate of water sprinklers (111). In an aversion testing research experiment, dairy cows were moved through a race towards a shower. The flow rate was set to either 0.4 L/min (1.1 kPa) or 4.5 L/min (8.9 kPa), and the ambient temperature was between 25 and 38°C. Cows moved more slowly through the race when the ambient temperature was greater, and although the cows lowered their heads when approaching the higher-pressure shower, they did not baulk or refuse to enter it.

In some systems it is common practice to handle cattle through a crush prior to slaughter, e.g., to check dentition or read ear tags. A study in the UK compared the post-stun responses and prevalence of ecchymoses (blood-splash) in cattle that had been handled through a crush prior to slaughter against those that had not (112). 788 animals were observed for post-stun responses, 466 of which had been restrained in a crush in the race immediately prior to entering the stunning box. They were stunned using a Jarvis electrical stun box with a cardiac arrest and spinal depolarisation cycles at 50 Hz AC, maximum current 3.5 A. When crush restraint was used, there was a significant increase in muscle tone ($P < 0.001$), rhythmic breathing ($P < 0.001$) and rotated eyeballs ($P < 0.004$) observed in the cradle, and a significant increase in limb movement at sticking ($P < 0.001$). 6061 carcases, half of which were processed using the crush prior to slaughter, were assessed for carcase defects. The group of carcases from animals that had been restrained in the crush prior to slaughter had significantly greater ($P \leq 0.001$) incidence of blood splash (also referred to as ecchymosis - small haemorrhages throughout the muscle tissue) than the group that had not been restrained in the crush (19.7% compared with 8.4%). The authors suggested that restraint in the crush immediately prior to entering the stun box was incurring stress, reducing welfare and contributing to incomplete stunning and associated carcase quality problems.

Low stress handling is advocated in all situations involving livestock. A study in an abattoir in Argentina compared two matching groups of 20 steers that had been lairaged overnight (113). One group, the 'low stress' group, was slaughtered first thing in the morning with minimal noise and disturbance, waiting for 20 min in the alley before entering the race, and the second group, the 'conventionally handled' group, was slaughtered next, under the normal processing conditions with no waiting in the alley, shouts from handlers and use of electric prods. The low stress group took 20% longer to process than the second group. Blood samples taken at exsanguination revealed higher plasma glucose, lactic acid and total protein concentrations in the conventionally handled group as compared to the low-stress group ($P < 0.05$), suggesting a poorer welfare condition. A similar study compared two groups of bulls taken from the same farm (114). One group was lairaged for 2 h and handled quietly to the stunning box. The second group was unloaded and driven back and forth along the alley for 30 min, by handlers that shouted and hit the bars of the pens with sticks, prior to slaughter. Electric goads were only used at the stun box. The quietly handled group received significantly less prods with the electric goad on entry to the stun box ($P < 0.03$), while the post mortem pH decline was faster in the second group, indicating a degree of activation of metabolic rate and 'excitement'. This was supported by significant increases in salivary cortisol concentration, and urinary cortisol and catecholamine concentrations in the second group compared to the quietly handled group. Likewise, a study in two cattle abattoirs in Australia identified a relationship between plasma cortisol concentration at exsanguination and measures of frequency of goad use, frequency of vocalisation or the head down behaviour in the animals (6). There is substantial evidence of the relationship between stockperson behaviour and animal behaviour (4, 5, 6, 9). A study across two abattoirs in Sweden showed a significant positive association between the counts of stockperson behaviours (contacting the animal with hand or with an implement; pushing; tail-twisting; electric goad) and counts of cattle behaviours (backing, turning, slipping, vocalisation; aggressive interaction) (115). There was also an effect of abattoir – behavioural counts (corrected for race length) were lower in a smaller mobile abattoir than in the larger fixed abattoir. In another study, the effect of using an electric goad, a rattle or manual urging on calf movement through a race or chute was compared in calves aged between 6 and 9 months (116). When the electric goad was used, calves ran more often and made contact with the sides of the race than when rattle or manual urging were used. However, when calves were handled using the electric goad, it was used much less often than rattle or manual urging were used when these were the handling aids available. It is not clear whether this is an effect of ability of calves to learn quickly from the aversive stimulus of the electric goad and therefore move forward more readily than when the rattle or manual urging was used, or whether there was an effect of the operators having a subconscious bias against frequent use the electric goad. The differences were significant only in intensively reared Holstein calves (which were also less fearful of the handlers), but similar trends were observed in extensively reared beef breeds. Interestingly, calves that had previously been handled using the electric goad learned that the buzzing noise was associated with goad use and one week later they would still move away when the goad was buzzed but not applied. The persistence of this memory was not evaluated, but if such a memory does persist, use of buzzers could facilitate animal movement if they had previously been handled using goads. In a study of vocalisation rate in 48 USA abattoirs (117), generally there were no significant differences in vocalisation rates between plants that did not use electric goads and those that did. However, when the researchers focused on plants that used the electric goad on 95% or more animals, there was a significant effect on vocalisation rates. In the high-goad-use plants, 15% of cattle vocalised, compared to 1.5% in plants that used no electric goads ($P < 0.01$).

In a study involving 150 stockpersons on farms in Brazil, it became clear that provision of formal training improved handling of cattle (118). The formal training was a three-day program involving both didactic and practical components, focused on animal-human interactions and animal welfare and was based on utilising animal behaviour to optimise animal handling. There was a strong emphasis on the fact that aversive handling negatively affects cattle behaviour and induces fear of humans. Scoring both stockperson and animal behaviour during cattle handling indicated that the non-trained stockpersons had the poorest quality of animal handling and the greatest incidence of undesirable animal behaviours, e.g., jumping, baulking, lying down, aggressive behaviour ($P < 0.05$). With these stockpersons, handling deteriorated during the working day ($P < 0.05$). In contrast, stockpersons that had been provided with formal training demonstrated higher quality of animal handling, had the greatest positive attitude scores and the lowest incidence of undesirable animal behaviours ($P < 0.05$). Handling did not deteriorate during the day.

Section summary - Handling techniques

It is clear that low-stress handling practices and training of stockpersons can have a significant positive effect on animal welfare and behaviour

CA2: Restraint

One study investigated the effect of restraint duration on plasma cortisol concentration in 1-2 month-old calves (119). The calves were manually restrained by leaning them against the pen wall. There were four treatment groups: blood collected immediately the calf was restrained; after 30 sec; 1 min; or 2 min. Cortisol concentrations were significantly greater in the 2 min group than in the other groups, the other groups did not differ from one another. The authors concluded that restraint for 2 min or more would affect the cortisol concentration in the sample. The results could be taken to indicate that restraint durations of less than 2 minutes are not stressful to calves, but that ignores the fact that there is a dynamic change in cortisol after a stressful event, that peaks at around 30 minutes, and there may be a delay between cortisol production being initiated and measurable change in the circulating blood.

A study on *Bos indicus* cattle in Mexico indicated that the stress of restraint in a squeeze chute could be reduced by fitting a mask on the animal’s face (120). The mask was described as “constructed of black velvet inside, a cushion in the middle and a leather exterior, in an elliptical form (60 cm x 20 cm). A thicker edge kept the body of the mask away from physical contact with the eye” and was fastened using elastic. Researchers measures heart rate, respiratory rate, behaviours during restraint and plasma cortisol concentration (blood sample taken after 3 min of restraint). Animals that had the mask fitted had lower heart rates and respiratory rates at the end of the 3 minutes than animals without the mask, lower agitation score and lower plasma cortisol. A similar study in Canada evaluated the use of a blindfold on 60 heifers handled in a cattle crush and 93 3-month-old calves restrained on a tilt table (121). Half the animals in each group were blindfolded using a dark-coloured towel secured over the eyes using a nylon rope halter. Blindfolded heifers exerted 23 – 28% less pressure on the neck bails than non-blindfolded heifers ($P < 0.05$); while blindfolded calves performed 44% less movements while on the tilt table than non-blindfolded caves ($P < 0.01$). Although application of the mask or blindfold itself could be challenging in a commercial situation and would restrict access to the frontal bone for mechanical or diathermic stunning, the technique may have value in calming an extremely agitated animal in the event of a short delay between restraint and stun.

Section summary - Restraint

Prolonged restraint is stressful to animals, but strategies to minimise the negative impact on animal welfare can be implemented, such as design of facilities appropriate to the animal size/class handled (Section GEN4: Restraint), restraining the animal immediately before stun application, and consideration of the use of a blindfold or mask to quieten agitated animals.

CA3: Stunning

CA3.1: Mechanical stunning

CA3.1.1: Shot position

A study on cadaver cattle heads investigated the effect of shot placement on physical damage to the brainstem (122). The researchers evaluated two placements: LOW (the intersection of two lines drawn from the top of the ear to the medial corner of the opposite eye; Figure 01A) or HIGH (midline position, halfway along a line drawn between the top of the poll and the level of the medial corners of the eyes; Figure 01B). Computer tomography was used to assess brain damage after application of a Cash Dispatch Kit cartridge-powered penetrating captive bolt device. In adult (> 2 y-o) heads, there was brainstem damage in 7 of 14 LOW position placements, and 18 of 18 HIGH position placements. In heads from cattle aged 6-24 months, brainstem damage was present in 11 of 19 LOW position and 13 of 16 HIGH position. In calf heads (< 1 month) all showed brainstem damage (11 HIGH and 14 LOW). The authors concluded that the HIGH position would reduce the risk of the animals regaining consciousness after stunning.

In the Czech Republic, a study of 627 cattle (bulls and cows) evaluated the effect of deviation from the ideal shot placement on induction of motor paralysis (collapse) (123). These researchers defined ideal as a midline position, halfway along a line drawn between the top of the poll and the level of the tops of the eyes (figure 01 C) – i.e., slightly above the position described as HIGH in the previous study. Stunning was carried out using a Termet Matador SS 3000 B cartridge-powered penetrative captive bolt instrument, on animals that were restrained in a stun box without neck or head restraint. Across all animals, failure to collapse occurred in 2.4% when the shot was applied within 3 cm of the ideal position, increasing to 72.2% when placement was greater than 7 cm from ideal ($P < 0.01$). The incidence of failure to collapse was higher in bulls than in cows ($P < 0.05$). The researchers concluded that from both animal welfare and operator safety perspectives, accurate placement of the stun was necessary.

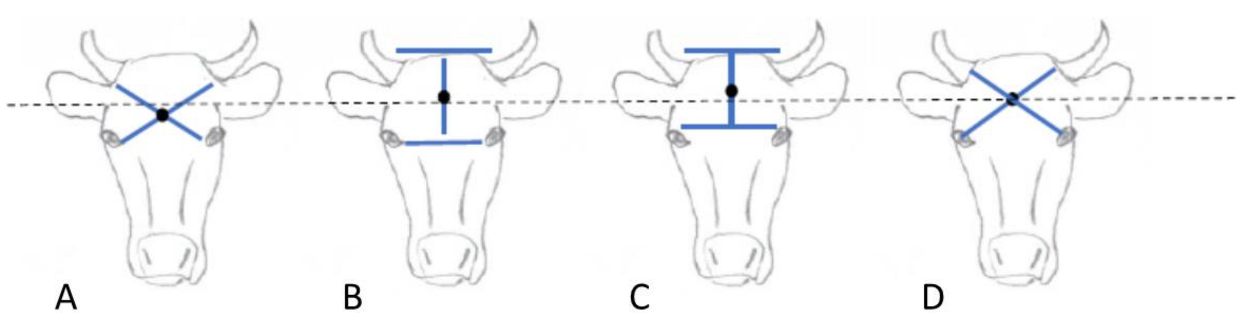


Figure 01: Alternative shot positions evaluated for cattle. A: LOW position assessed by (122); B:, HIGH position assessed by (122); C: 'ideal' position defined by ref (123); D: recommended position based on the Model Code of Practice (62). Dotted horizontal line indicates level as defined by Model Code of Practice.

In a separate study, the same researchers hypothesised that slaughterperson skill levels would be related to the incidence of poor shot placement and failure to collapse in cattle (124). They monitored stunning in two Czech abattoirs, and examined 382 heads at one (abattoir A) and 235 heads at the other (Abattoir B). Both abattoirs used similar stunning restraint and apparatus (no head restraint, and the Matador S 3000 B captive bolt). More animals were shot outside the 3 cm radius of ideal in abattoir A than in abattoir B ($P < 0.0001$), and more animals failed to collapse on first shot in abattoir A ($P < 0.05$). The differences were more marked in bulls than in cows and heifers. The researchers concluded that the slaughterperson in abattoir B was more skilled than in abattoir A based on shot position, use of restuns and failure to collapse, but failed to show a correlation with other indicators of ineffective stun (eye reflexes, rhythmic breathing, righting reflex, vocalisation).

A study of 100 cattle in South Africa indicated that multiple application of the captive bolt stunner (details of instrument not reported) may lead to increased plasma cortisol or heat shock proteins in cattle (104). However, the data presented are confounded by transport duration, which also had an effect on these parameters.

The potential efficacy of an occipital (just behind the poll) application of penetrating or non-penetrating captive bolt (Cash Special 0.25 calibre cartridge powered device) was evaluated in 12 sedated calves (6 in assigned to each treatment) (125). The calves were approximately 5 months old and 100-200 kg liveweight. Based on EEG and behavioural responses, all calves were effectively stunned immediately on application of the instrument (loss of corneal reflex, fixed central eye and loss of righting reflex), although the heart continued to function for up to 5 minutes (after which the calves were chemically euthanased), and respiration continued for up to 2 minutes in one calf. The authors concluded that the occipital approach was suitable for euthanasing this size of animal, as long as a secondary kill step (e.g., exsanguination) was also performed within 5 minutes of stunning.

CA3.1.2: Efficacy of cartridge-powered penetrative captive bolt devices

In a survey of 850 cattle processed through an abattoir in Portugal, the efficacy of penetrative captive bolt stunning (using a cartridge driven CASH® Cowpuncher device) decreased as the age of animal increased (126). Stunning efficiency of young animals (< 12 months-old) was 89.1%, but only 50.3% in animals over 30 months of age. Overall efficacy was 68.2%. Efficacy was lower in females (63.1%) than males (75.6%) overall, but this was confounded by age: in animals over 12 months, efficacy was greater in females than males. Efficacy was also greater in dairy breed cattle than in beef breeds. The conditions did not reflect modern Australian processing, in that the restraint box used did not have head or body restraint. It is likely that application of the shot at the correct position was more difficult than if head or body restraint were used.

CA3.1.3: Efficacy of cartridge-powered non-penetrative captive bolt devices

A study involving 90 6-8 month-old veal calves investigated the need to apply a secondary kill step (electrically induced cardiac arrest) after use of a non-penetrating captive bolt instrument (127). All calves were effectively stunned by the non-penetrating captive bolt, based on loss of rhythmic breathing and loss of reflexes, leading the authors to conclude that the cardiac arrest step was not necessary from a welfare aspect.

Ten calves were involved in a detailed study into the electroencephalographic (EEG) changes associated with non-penetrating captive bolt application (128). The calves (109-144 kg, mixed-breed) were lightly anaesthetised to allow detailed EEG collection without the overlay of noise, light or muscle movement artefact, and EEG was recorded pre and post captive bolt application. There was a significant change in EEG parameters, assessed both qualitatively and by quantitative comparison of total Power (Ptot) after application of the stun. the authors concluded that these changes would be sufficient to produce insensibility within 14 seconds of the application to conscious animals.

CA3.1.4: Pneumatic-powered devices

Pneumatically powered (air-powered) captive bolt instruments are commonplace in modern high-throughput beef processing. Much of pre-2010 published research utilised cartridge-driven instruments, so there has been interest recently in validating the use of the pneumatically driven instruments. A study involving 42 Nelore (*Bos indicus*) cattle, over 400 kg liveweight evaluated the performance of the Jarvis USSS-1 pneumatically powered penetrative captive bolt instrument set at 160, 175 or 190 psi, and the Jarvis USSS-2A pneumatically powered non-penetrative captive bolt instrument, set at 220 psi (129). The shots were applied at the intersection of two imaginary lines drawn from the base of the horn to the corner of the opposite eye, and all cattle were confirmed stunned after a single shot. Heads were frozen, then split on a sagittal plane, ensuring that the split passed though the bolt hole or impact point, and evaluated by physical inspection and some were evaluated using x-radiography. For the penetrating captive bolt, two of the heads shot at 160 psi did not have full-thickness penetration of the skull. Bolt penetration depth was greatest in the head shot at 190 psi. The area of haemorrhage over the brain was greatest for the penetrating captive bolt at 190 psi, while the number of heads showing blot clots over the upper surface of the brain was highest for the non-penetrating captive bolt (100% compared to 70-83%). Haemorrhage over the brainstem structures was more frequent with the penetrating captive bolt (10 - 50%) than with the non-penetrating device (0%). The authors concluded that use of the non-penetrating device failed to produce sufficient damage to the brainstem structures for reliable stunning, and only the higher setting of 190 psi with the penetrative instrument provided damage to the thalamus and brainstem. They recommended that airline pressures below 190 psi should not be used for stunning of adult cattle. This team of researchers also carried out two larger scale studies evaluating the performance of the instruments in a commercial setting. In the first study, 443 Zebu (*Bos indicus*) or crossbred cows and bulls were stunned using the Jarvis USSS-1 pneumatically powered penetrative captive bolt instrument, set at 160-175 psi (n = 82) or 190 psi (n = 363) (130). Animals stunned at the higher airline pressure showed less instances of rhythmic breathing (8% vs 27%), an indicator of incomplete stunning; greater instances of relaxed jaw (48% vs 22%) and greater incidences of tongue protrusion (12% vs 4%), the latter two of which are considered to be indicators of unconsciousness. Again, the recommendation was that airline pressures below 190 psi should not be used when processing mature cattle. The second study considered Zebu (*Bos indicus*) or crossbred bulls, steers and cows over the age of 20 months (131). In this study, 92 animals were stunned using the Jarvis USSS-2A pneumatically powered non-penetrative captive bolt instrument, set at 210-220 psi; and 363 were stunned using the Jarvis USSS-1 pneumatically powered penetrative captive bolt instrument, set at 190 psi. In the group of cattle shot with the penetrating device, 99% collapsed on first shot, compared with 91% of the non-penetrating group ($P < 0.002$). The incidence of eyeball rotation, righting reflex and response to nostril stimulation (indicators of ineffective stun) were greater ($P < 0.001$) in the non-penetrative group (5%, 7% and 2% respectively) than in the penetrative group (1% for eyeball rotation and righting reflex, 0% for nostril reflex). The researchers concluded that use of the penetrative captive bolt set at

190 psi was more effective in stunning the animals than the non-penetrative instrument set at 210-220 psi. This was attributed to the differences in physical parameters of the bolts, which were objectively measured in the study. Bolt velocity, momentum, kinetic energy, energy density and sectional density were all significantly lower for the non-penetrative device set at 210-220 psi than for the penetrative device set at 190 psi ($P < 0.001$).

Researchers compared the effects of the pneumatically-powered penetrating captive bolt devices, Jarvis USSS-1 and USSS-21 on stunning-related variables in cattle (132). The instruments were applied once or twice, and operated at 190-198 psi. The efficacy of stun was 97-98% regardless of instrument used or number of applications (over 500 slaughter age cattle in each treatment group). Double application led to greater skull and brain tissue damage than single application ($P < 0.01$, 100 heads in each treatment group examined), while the USSS-21 instrument led to a greater incidence of brainstem damage ($P < 0.01$). The authors concluded that although the instruments' performance was essentially similar, the USSS-21 instrument might have a slight advantage in terms of ability to cause brainstem damage. An electroencephalographic assessment of the performance of the Jarvis USSS-1 pneumatically powered penetrating captive bolt and the Jarvis USSS-2A pneumatically powered non-penetrating captive bolt in adult (30 month-old, > 550 kg liveweight) Zebu (*Bos indicus*) bulls in Brazil indicated that although the penetrative captive bolt rendered all bulls unconscious, the efficacy of the non-penetrative instrument was limited, with 2 of 11 bulls showing evidence of incomplete concussion (133). Both instruments were set to 220 psi. 20 bulls were stunned using the penetrative captive bolt instrument and all had EEG waveforms that were incompatible with consciousness. Immediately after application, the EEG showed movement artefact followed by transitional phase (mean duration 9.1 s) before becoming isoelectric. Onset of isoelectric waveform ranged between 1 and 27 s (mean 11.6 s). For the 11 bulls stunned using the non-penetrative device, the mean duration of the transitional phase was 13 s, and mean onset of the isoelectric state was 8.2 s (range 1-26 s), but in two animals, normal EEG patterns were seen in two animals, lasting 20 s in one and 3 s in the other, after which a back-up stun was applied. High Amplitude Low Frequency (HALF) activity (an intermediate state with regard to consciousness) was seen in 5 animals in each group, either before or after the transitional phase. A study in Brazil assessed indicators of unconsciousness (absence of rhythmic breathing, absence of spontaneous blinking, absence of eye reflexes and absence of response to pinch) in 434 *Bos indicus* bulls (90). In this study, 279 were stunned using a pneumatically-powered penetrative captive bolt (Jarvis brand, 180-190 psi) followed by thoracic sticking; 67 were stunned using a non-penetrative pneumatically-powered captive bolt (Jarvis brand, 160-170 psi) followed by neck cutting (with the remaining 88 slaughtered by neck cutting without prior stunning). The stunned animals were restrained in an upright position with neck yoke and chin lift. Repeat application was required in 46% of the animals stunned using the non-penetrative instrument , compared to only 2% of animals stunned using the penetrative instrument ($P < 0.001$). There were no significant differences between stun methods in the presence of indicators of ineffective stunning (< 1% of animals) at 20 s and 60 s post exsanguination.

CA3.1.5: Penetrative captive bolt length

There has been some interest in the implications of altering the length of the penetrative captive bolt. A study involving 45 *Bos taurus* grain fed cattle divided into three groups compared standard (15.2 cm), medium (16.5 cm) and long (17.8 cm) bolts fitted into a Jarvis USSS-1 pneumatically-powered captive bolt device (134). Video footage was used to assess the degree of post stun kicking while on the bleed rail, and the heads were examined for brain damage. The medium bolt length tended to result in less kicking (5 kicks in the 10 sec observation window) than the other bolt lengths (both 6 kicks), which could improve operator safety. All animals were rendered unconscious, based on lack of righting reflex, and the degree of brain damage increased as bolt length increased (more detail on the degree of damage to various parts of the brain were reported separately (135)). None of the heads showed physical disruption of the brainstem, indicating that the secondary kill step of exsanguination was vital to ensure rapid death. A similar study by the same research team found that increasing bolt length did not influence hind limb activity, but there was a breed effect – Holstein cattle exhibited significantly more hind limb kicks that non-Holstein cattle (136).

Section summary - Mechanical stunning

Penetrative mechanical stunning is highly effective when applied in the correct position. The correct position as recommended by the current MCoP, or marginally higher appears to be most appropriate. The accuracy of the shot becomes more critical as animals mature, and also when using non-penetrative mechanical stunners. Use of head restraint will improve shot accuracy. Non-penetrative stunning has a lower first-shot efficacy than penetrative mechanical stunning. Modern pneumatically-powered devices are as effective, and may be more so, as conventional cartridge-driven instruments.

CA3.2: Electrical stunning

The Jarvis beef stunner was introduced to the UK in the late 1990s, and a study was carried out to validate its efficacy (137). The apparatus used in the study delivered thee cycles (head-only; cardiac arrest and spinal discharge) of current at 550V, 50 Hz with a maximum current of 3.5 A. Three experiments were carried out involving 92 *Bos taurus* cattle (300 – 500 kg liveweight). In the first experiment, 67 cattle were used to determine the minimum current required to achieve effective stunning, measured using EEG. Variations in current applied were achieved by altering the applied voltage in the system, and a range of 0.46 – 3.57 A were applied for a period of 3 s. All animals were effectively stunned based on the presence of epileptiform activity in the EEG. Subsequently, 64 of these animals were restunned for Experiment 2, adding a cardiac arrest cycle of 5 s duration, and electrocardiogram (ECG) was measured. Currents of greater than 1.51 A were sufficient to stop the heart. In the third experiment, the aim was to identify the minimum current required to produce an effective stun, if the current was applied for < 1 s, instead of the 3 s application used in experiment 1. There were 25 cattle enrolled into this study, and after stunning they were rolled out onto a straw bed and allowed to recover. Stunning was considered effective if the animals showed loss of posture, loss of rhythmic breathing, loss of eye reflexes and the tonic-clonic progression of an epileptic fit. Currents of > 1.51 A induced an effective stun. Both tonic and clonic phases were variable in duration (1 – 23 s and 1`-87 s respectively), and in 17 animals rhythmic breathing returned after 17 – 87 s. The researchers concluded that the apparatus could effectively stun cattle but recommended that the cardiac arrest cycle was used to ensure that animals would not return to consciousness while bleeding out. Early models of the Jarvis beef stunner placed the cardiac arrest electrode at the brisket. A study published in 2009 investigated the potential for using a chest electrode in place of the brisket electrode, i.e., moving it further back along the sternum of the animal (138). The measures investigated were incidence of cardiac arrest (electrocardiogram), post-stun movement and incidence of carcass ecchymoses (blood splash). In this study, 287 cattle (250 – 450 kg liveweight) were processed: 40 using the brisket electrode and a three-cycle setting – head; cardiac arrest, spinal discharge; 40 using the chest electrode and a three-cycle setting; and 207 using the chest electrode and a two-cycle setting – head, cardiac arrest, without the spinal discharge in order to evaluate post-stun movements. There were no differences between groups in terms of induction of cardiac arrest, post-stun responses or broken bones. However, use of the chest electrode significantly reduced the incidence of blood splash in the sirloin muscle from 50 – 58% to 30 – 37% (P < 0.05). Subsequently, the chest electrode has been fitted to all Jarvis electrical stun units, such that the full stun setting with cardiac arrest includes both brisket and chest contact electrodes. (Allan Lee, Jarvis Engineering, personal communication).

A pulsed ultra-high current electrical stunning system was evaluated on 97 steers (500 – 600 kg liveweight) across three experiments. In the first experiment (92), 40 steers were stunned using 70 A, 5000 V for 50 ms and behavioural responses to the stun were assessed. All animals collapsed, either onto the floor of the stun box, or propped against the side walls in a tonic or shuddering phase. This postural rigidity prevented rapid extraction of the body onto the bleed table. Five animals remained in a flaccid, relaxed catatonic phase with no physical movements. Behaviours observed included shuddering, paddling or 'swimming' motions, kicking, arched back, ear twitch, eyelid flutter and gaping of the mouth. The second experiment occurred after modifications to the apparatus to improve application of the current and improve ease of removal of the body from the stunning box. Thirteen steers were used to assess the duration of insensibility, monitored until signs for returning rhythmic breathing, corneal and palpebral reflexes at which point they were stunned using a non-penetrative captive bolt. All animals collapsed immediately after the electric stun, and this was very rapidly followed by a convulsive, jerky kicking phase. This lasted for 5.3-14.1 s, then the animals entered a relaxed stationary phase. Rhythmic breathing returned at 23-32 s after stun, and eye reflexes returned on average after 31 s (max 63 s). Heart rate was increased after the stun. A second group of 25 steers were then used to assess the return of reflexes under processing conditions, whereby the neck cut was performed, the oesophagus was clipped and the body was shackled and hoisted. Six animals required back-up stunning while on the bleed rail (56 – 201 s post-stun), while another 6 showed a transient period of spontaneous blinking or eye reflexes while on the bleed table and no other signs of recovery. In the third experiment, 19 cattle were prepared for electrocorticogram (ECoG) measurement of baseline and post-stun EEG activity including visually evoked responses (VER). Four data sets were poor quality (technical problems) so they were excluded from further analysis. The first 10-15 s of post-stun recording were discarded as they were heavily contaminated with movement artefact while leads were re-attached. The amplitude of the EEG was significantly reduced after stunning (apart from one animal which showed both EEG and behavioural evidence of a Grand Mal epileptiform seizure). The amplitude then gradually increased but had not reached baseline before the experiment was terminated by application of a captive bolt (4 min after stunning). VERs could not be identified post stunning. The authors concluded that pulsed ultra-high current stunning does not rely on an induced epileptiform state to induce or maintain insensibility and recommended that the technology be further developed as a potential option for commercial Halal slaughter. Although there is no further published material, further pre-commercial development is proceeding in the EU (S. Wotton, personal communication).

Section summary - Electrical stunning

Electrical stunning of cattle is widely used in the Australian industry, particularly for cull cows. There are some concerns in terms of meat quality attributes when used in feedlot cattle, and, in the case of head-only electrical stunning, the processing set-up must achieve a short stun-to-stick interval to ensure that animals do not regain consciousness while exsanguinating. Despite its popularity, there is a paucity of published literature on electrical stunning of cattle, particularly in light of recent developments in electrical stunning of other species (e.g., pigs and poultry) in which alternative frequencies and/or waveforms have shown promise (See sections PG3.2 and PO2.2).

CA3.3: Application of microwave energy - DTS: Diathermic Syncope®

The DTS: Diathermic Syncope® system uses microwave energy to heat the brain to a state of hyperthermic unconsciousness (> 43°C). Development work included application of microwave energy at 922 MHz to 20 cadaver cattle heads (refrigerated and treated within two days of slaughter) to determine the heating profile of the brain; and also to develop incident power/time predictions for different liveweights of cattle (139). For temperature profiling, fibreoptic thermoprobes were inserted at five different locations on the head surface (frontal skin directly under the applicator; neck skin behind the jaw; under the skin at the base of the ear; subconjunctival of an eye) and at five locations within the brain (frontal upper cerebrum in line with the applicator; occipital cerebral area under the poll; centre of the brain mass; base of the brain in the region of the hypothalamus; brainstem/reticular formation). The temperature profiling used a 4 kW application for 10 s on eight heads. There was no significant heating at the neck, eye or nose probes. Temperatures increased by 3.66 °C (range 1.5 – 8 °C) in the lower parts of the brain, by 6.42 °C (range 3 – 17 °C) in the upper parts of the brain and by 5.93 °C (range 0.2 – 9 °C) in the skin directly below the applicator. To develop a predictive model for power/time requirements in different liveweights of cattle, 12 heads from animals of known liveweight were used. Energy applications ranged from 2.5 – 9 kW, with exposure times of 15-60 s. Heating rates in each area of tissue were found to be linear, with heating rates higher at the upper surfaces of the brain than in the deeper sections. The data indicated that microwave energy could be used to raise the brain temperature to a level that would induce unconsciousness, while optimisation of the energy transfer would be recommended to improve penetration into the brain tissue. Based on the findings of the laboratory study, microwave energy application was evaluated on nine anaesthetised cattle (*Bos taurus* females, approximately 180 kg liveweight) (140). Subdermal electroencephalogram (EEG) needles were implanted to allow EEG data collection before and after energy application. Two animals received 20 kW for 15 s; two received 20 kW for 10 s; three received 30 kW for 10 s; one received 30 kW for 5 s; and one received 12 kW for 25 s. All energy applications resulted in EEG patterns indicative of seizure-like activity, a state that is not compatible with consciousness. It appeared that shorter durations of application led to faster changes in the EEG, while increasing the power of application led to a longer duration of EEG changes, but the numbers of animals included in the study were small, so individual variation may have contributed to this impression. The low power (12 kW) application was associated with a very slow time to onset of changes in the EEG, so was not recommended for further study. For the other applications, time to onset of EEG changes was 4-22 s, and all treatments suppressed the EEG for at least 37 s and up to more than 162 s (when recording was stopped). Heart rate was also recorded in this study: heart rate dropped within 5 s after the onset of energy application, rebounded after 23 s and stabilised after 160 s. The final heart rate was lower than the pre-application baseline, but the difference was not statistically significant. The authors concluded that the outcomes were promising for commercial implementation with further development and validation of application energy parameters. Two pilot scale evaluations of the DTS: Diathermic Syncope system for stunning conscious cattle have been conducted (141). In the first study, ten Angus heifers (350 – 400 kg liveweight) received microwave energy application. A range of energy applications were applied, grouped as HIGH (> 290 kJ applied, n = 3), MED (200 < 290 kJ, n = 3) and LOW (< 200 kJ, n = 4). EEG data were collected from animals prior to stunning and for at least 2 min post stunning. Live behavioural observations were made until evidence of returning eye reflexes was detected (at which point a back-up captive bolt stun was applied), or for 7 minutes, whichever occurred first. Video recordings were used to fully characterise the behavioural response. The second study used the same methodologies as the first and was carried out after refinements to the energy delivery system. It involved 20 crossbred *Bos taurus* cows (liveweight 350 – 400 kg), receiving energy applications in the range 200 – 360 kJ. In the first study, one animal that received 38.55 kJ did not fall unconscious, while all others (45.87 kJ and above) were deemed unconscious based on loss of posture, loss of corneal reflex and fixed, staring eye. Corneal reflexes returned in two animals (217-139 kJ) about 4 minutes after loss of posture. During study 2, all but 3 animals (in which there had been technical problems with energy delivery) were deemed unconscious after energy application. Energy application resulted in eyelid flicker, uncontrolled blinking, nystagmus (eyes twitching from side to side). The animals initially lost posture, but quickly gained a tonic rigid posture similar to that described in relation to the pulsed ultra-high current stun. This tonic posture restricted extraction of the body from the restraint unit. In this tonic phase, the eye was fixed and staring with no response to movement and no pupillary response to light. Slow, deep, rhythmic breathing was present. Animals that received 275 kJ and above were rendered catatonic or dead and the bodies were flaccid. EEG data were similar to the EEG data collected from the anaesthetised animals in the study described above, with epileptiform changes evident in the stunned animals. In four animals (85, 225, 225 and 275 kJ applications) these changes began to resolve after 80 – 160 s after energy application. Reduced amplitude or isoelectric EEG was evident in the catatonic or dead animals. The authors concluded that microwave energy application using the DTS: Diathermic Syncope system could render cattle unconscious for a sufficient duration to allow exsanguination, while evidence of returning reflexes indicated that the insensibility might be recoverable. Further development to optimize energy delivery and understand the critical limits of energy application parameters for a wide range of cattle was recommended.

Section summary - DTS

The DTS:Diathermic Syncope(R) shows promise as a potential alternative to slaughter without stunning for the Halal and Kosher markets. An application has been lodged with DAWR to authorise the technology as an approved method of stunning cattle prior to slaughter (James Ralph, personal communication)

CA3.4: Carbon dioxide stunning

Carbon dioxide (CO_2) was used to stun 20 Koran Hanwoo steers (26-30 months old) (142). The steers were in a chamber containing 70% CO_2 for 140 s. The study focused on meat quality characteristics, compared to carcases from similar steers stunned using a captive bolt instrument (presumably penetrative, but not detailed), and no behavioural or physiological measures were taken. pHu was lower in the CO_2 carcases than captive bolt, which could suggest an increase in metabolic rate or acute 'stress'/'excitation' associated with the captive bolt stunning process. When the steers were classified by liveweight (620-710 kg or 720-760 kg), the heavier animals showed lower pHu than the lighter animals across either stun treatment, while tenderness was improved in the heavier animals stunned using CO_2.

CA3.5: Puntilla

Although puntilla (a stab into the back of the neck to sever the spinal cord) is not recommended as a slaughter method by the OIE, it is still used in many developing countries. A study in Bolivia evaluated 309 cattle (steer and heifers, weighing between 200 and 580 kg) slaughtered using the puntilla method (143, 144). In this study, 24% of animals were stabbed more than once, up to 8 times before they collapsed. Heavier animals were more likely to require repeat stabbing; and experience of the operator was also a significant influence on number of stabs. After collapse, 92% of animals showed indicators associated with brain and spinal function (rhythmic breathing, rotated eyeballs, nystagmus and positive blink and pupillary reflex), and 22% attempted to stand.

This study confirms the negative welfare consequences of the puntilla method, demonstrating that the animals are likely to remain conscious.

CA4: Bleeding

CA4.1: Methodology

Cattle are exsanguinated either using the thoracic sticking method (the knife is inserted into the base of the neck, close to the thoracic inlet, severing the common carotid artery as it arises from the brachiocephalic trunk), or using a transverse neck cut below the jaw (severing the carotid arteries and jugular veins). The transverse neck cut is preferred by the halal and kosher markets, as it aligns with the descriptions in the religious texts. However, from an animal welfare point of view, the thoracic sticking method is preferable. Mammals have an alternative blood supply to the brain via anastomoses from the carotid arteries to the vertebral arteries which supply the rete mirabilis and basi-occipital plexus perfusing the brain. This alternative perfusion pathway is sufficient to maintain consciousness in some animals, despite both carotid arteries being severed by the transverse neck cut. Furthermore, thoracic sticking technique avoids the issues associated with the development of false aneurysms (section 7.4.3) See section Gen7.4: Slaughter without stunning for further detail.

CA4.2: Time to brain death

As a result of the alternative prefusion of the brain, the time to loss of consciousness and brain death in cattle can be variable. In animals slaughtered without prior stunning, time to collapse has been recorded to range from 20 to greater than 60 s, and brainstem reflexes have been recorded at 100-120 s after sticking (84, 85). See Gen6.4: Slaughter without stunning for further detail.

CA5: Animal welfare assessment

CA5.1: Assessment of lairage and handling

Vocalisation scoring can be used to assess the impacts of alterations to handling equipment. In a study of five abattoirs, vocalisations were scored before and after modifications, and a chi-square test used to compare the vocalisation rates (117). Significant reductions in vocalisation rates were seen after: reducing the voltage of the electric goad; installing lighting at the centre-track conveyor to reduce baulking; and replacing a v-restrainer conveyor with a centre-track conveyor. Reductions in vocalisation were seen after installation of a false floor below a centre-track conveyor and reducing the pressure applied by the neck bails – these reductions were not statistically significant due to small numbers of animals being observed (although a Fishers exact test may have assisted in the analysis in these cases). Infra-red thermography of the eye (Ocular IRT) has been proposed as an assessment measure. In a study of 175 bulls in Spain, the mean Ocular IRT values measured immediately before slaughter were correlated ($r=0.66$, $P < 0.01$) with pHu in lighter animals (< 250 kg liveweight) (145). Ocular IRT could predict the likelihood of high pHu, and so was an indicator of stress. With further study, Ocular IRT could be used as a selection tool, allowing animals to be selected for further lairage rest.

CA5.1: Post-stun responses after mechanical stunning

After stunning, and during bleeding many cattle show movements such as kicking, paddling or neck arching. A French study examined the relationships between indicators of unconsciousness (collapse, loss of rhythmic breathing, loss of eye movements and reflexes), shot position and angle, head shape and post-stun movements in cattle that had been stunned using a Termet Super Securit 3000 cartridge-powered penetrative captive bolt instrument (146). Animals were either processed as normal (shackled, hoisted and then bled using the thoracic stick method), or after verification of unconsciousness, the spinal cord was severed using the puntilla method before shackling. Post-stun movements were recorded in 16 of 20 bulls and 56 of 58 cows and could persist for up to 3 min post stun. There was a negative correlation between post stun movements and stun-stick interval. Stun-stick intervals in the study were on average 97.1 s. A high shot was associated with greater front leg paddling. Severance of the spinal cord did not alter post-stun movements. The authors concluded that there may be relationships between post-stun movements, shot placements and initial bleeding efficiency but the underlying mechanisms were not clear.

A number of studies have been conducted to validate and understand the factors underlying the various indicators used to assess effectiveness of mechanical stunning. In the Czech Republic, the post-stun behaviours and reflexes following penetrative captive bolt stunning using a Termet Matador SS 3000 B cartridge-driven instrument were characterised in 355 cows and heifers (13 – 201 months-old, 495 – 515 kg liveweight) and 262 bulls (12 – 92 months, 725 – 745 kg) across two abattoirs (147). Neither facility used head or body restraint. In 79.6% of animals, the shot placement was more than 2 cm away from the ideal position. The researchers found that certain indicators of ineffective stunning were more commonly seen in females than bulls (nystagmus and spontaneous limb movements, both $P < 0.05$), while others were more commonly seen in bulls ($P < 0.05$; vocalisation, presence of rhythmic breathing, rotated eyeballs, eye reflexes, vocalisation and non-protruding tongue). There was no relationship detected between shot placement (distance from the ideal position) and occurrence of indicators of ineffective stunning – in fact it appeared that the frequency of these indicators increased as distance from ideal increased up to 3 – 4 cm, but then decreased again. However, when the study is read in detail, some animals were re-stunned if they failed to collapse on first shot, and the measurements of distance were taken based on the first shot. It is likely that animals for whom the first shot was greater than 3 – 4 cm away from ideal received a second shot, and if this second shot were closer to the ideal position, the data on post-stun indicators would be confounded by the response to the second shot. The researchers recommended the use of head restraint.

In a Portuguese study of 850 cattle stunned using a penetrative captive bolt, improperly stunned animals showed: muscle tone of the ears (17.8%), no tonic phase (11.5%), presence of rhythmic breathing (9.4%), and vocalisation (7.9%) (126). Animals that failed to collapse immediately, showed rotated eyeballs, rhythmic breathing and flinch response to a pinch of the ear or nose were more likely to also show signs of recovery (e.g., returning corneal reflex, returning righting reflex).

Buffalo

BU1: Pre-slaughter handling

Literature pertaining to bison (*Bison bison*) was considered in addition to literature pertaining to buffalo (*Bubalus bubalis*), as bison is a large rangeland species for which handling challenges may be similar to those encountered when handling rangeland buffalo.

There is a paucity of published literature on buffalo and bison handling and restraint, while there is a growing body of research on the management of dairy buffalo. In dairy buffalo weaner calves, increased aggression and reductions in time spent lying were observed under higher stocking densities (148). The researchers estimated body surface area based on liveweight and gave groups of 10 weaners either 50% or 90% of their body surface area as space allowance. Calves in the 50% group spent more time standing ($P < 0.001$) and performed more agonistic interactions ($P < 0.01$) than calves in the 90% group. They concluded that the space allowance of 50% of body surface area may was likely to be inadequate.

Rangeland bison can appear calm and docile when at pasture, but handling can induce fear, aggression and stampeding. Observations of the routine handling of bison (50), described licking, blinking, huddling, raised tail, circling, backing up and baulking as indicators of stress or fear, and recommended handling in races with solid walls. The researchers also noted that bison do not easily follow one another in a race and may climb over one another. Similarly, water buffalo can also appear calm and docile, but when provoked or stressed even well-handled dairy buffalo can revert to feral behaviour patterns and demonstrate aggressive or defensive behaviour (149). A study of 100 buffalo slaughtered in India indicated that male animals were more likely to be aggressive than females and reported that blindfolds are used on nervous animals to assist in handling (150).

In a review of the welfare of water buffaloes during the slaughter process (13) it was noted that if loading ramps are steeper than 17.6% from the horizontal, foot battens should be installed to reduce the risk of slipping, and that passageways and raceways should be wide enough to allow the animal to move freely, stating that the use of narrow chutes designed for cattle can increase falls and collisions when handling larger (> 600 kg liveweight) buffalo. These authors also commented that restraining buffalo in equipment designed for cattle can be challenging due to differences in body shape, including short, thick necks and long horns in buffalo.

A study compared three groups of farmed bison – one shot in a corral after mustering, one transported and lairaged for 18 h and a third that were processed through a local commercial abattoir having been sourced from a single farm and lairaged for 9 h (151). Details on transport duration for the third group was not given, but it was stated that a mixed auction mob including a bull was in the adjacent pen and cattle were also present at the abattoir which could have increased stress levels in the bison. The first two groups were handled through a race and squeeze-chute and blood sampled, before being either released into the corral for shooting or into a corral for loading onto the truck. The third group were rifle-shot in a corral at the abattoir and blood was collected from the flowing exsanguinate. Carcases from the on-farm slaughtered group had least bruising; while those that had been rested for 18 h had the lowest plasma cortisol concentration. The authors did not speculate the source of bruising in groups 2 and 3, and concluded that on-farm slaughter would be optimal, followed by provision of an 18 h lairage period after transportation.

BU2: Restraint

Head restraint in the stunning box is recommended to reduce the time spent in the restraint, and to increase the accuracy of stun placement. A Colombian study found that where head restraint was used, a reduced number of shots were required to stun the buffalo, compared to when no head restraint was used (up to 3 shots versus up to 8 shots).

BU3: Stunning

BU3.1: Mechanical stunning

Buffalo have large frontal sinuses in the skull, which limit the use of captive bolt instruments applied at the frontal position due to absorption of the kinetic energy applied. For that reason, the majority of buffalo processing uses free bullet shooting, although recent development of high-powered captive bolt instruments have allowed effective frontal-position stunning of buffalo. A study to validate the use of a specially developed bullet casing gun (with 357 Mag/10.2 g hollow point bullets) for stunning water buffaloes was undertaken (152). In 20 buffalo (aged between 1 and 9 years), 19 were effectively stunned, while a single 9-year-old bull did not immediately collapse and was re-stunned. This instrument does not yet appear to be commercially available.

Stunning efficacy using a penetrative captive bolt device, as affected by shot position, was studied in water buffalo of between 350 and 700 kg liveweight (153). Neither frontal (n = 1) nor crown (n = 3) application of a penetrating captive bolt resulted in effective stunning of sufficient depth to allow exsanguination. In 30 animals in which the penetrating captive bolt was applied at the poll position, 29 collapsed immediately, with the remaining animals collapsing after about 5 s. Following stunning, 16 of the animals were deemed to have shallow depth of concussion based on return of reflexes or eyeball rotation or nystagmus. The authors recommended that sticking occurred promptly to ensure that animals do not recover consciousness during bleed-out.

As buffalo is a desirable meat for the halal market, a study was undertaken to evaluate the effectiveness of a non-penetrating captive bolt, positioned at the crown or poll position (154). The crown position was effective in small animals (≤ 89.6 kg carcass weight), and the poll position was effective in animals up to 183.6 kg carcass weight (~353 kg liveweight). In larger animals, efficacy was influenced by accuracy of application, as access to the poll can be limited by the behaviour of buffalo, who tend to tip their noses upwards and conceal the target application point.

BU4: Bleeding

Bleeding of buffalo is either by the halal neck cut or by the thoracic sticking method. Although there is little published research on the topic of loss of consciousness, a survey of the development of false aneurysms in buffalo slaughtered without prior stunning found large (> 3 cm) false aneurysms in 4 of 11 (36%) of buffalo, as compared with 17% of cattle in similar facilities (89). The authors also noted that the slaughtermen faced a challenge when exsanguinating the buffalo in that horns prevented the neck from being twisted to present the ventral surface uppermost for the neck cut (the animals were cast in lateral recumbency). A further description of buffalo slaughter in Bangladesh (155) described that the time from neck cutting to death (based on absence of visible or palpable pumping of the heart) was 7 - 8 min. It is important to note that most scientific studies use brain function as an indicator of consciousness or death, rather than heart function: the heart may continue to beat for a period of time after clinical brain death, dependant on the cause of brain death.

BU5: Animal welfare assessment

A study on the welfare of water buffalo in four Bangladeshi slaughterhouses (156) used a basic set of animal-based measures to assess welfare during lairage, casting (restraint for slaughter) and slaughter. After arrival, the following animal-based measures were used: Signs of dehydration, the presence of skin injuries and/or oculo-nasal discharge, subjective assessment of the animal's health condition. During casting and restraint, the occurrence of new injuries following casting and vocalization were recorded. The number of cuts made to the neck during slaughter and any stabbing prior to severing the carotid arteries was used in the assessment of slaughter. The effectiveness of throat cut and subsequent loss of consciousness was measured using palpebral and corneal reflexes, and absence of breathing.

Pigs

PG1: Pre-slaughter handling

PG1.1: Arrival

The arrival of pigs at the abattoir represents the first process in the pre-slaughter period. Pig condition at arrival and unloading is determined by the impact of the production process, loading on the farm, transport conditions (including the physical features of the truck, mixing of animals and thermal environment) and duration or transport (157). A study of pigs transported at different densities for a period of around 8 hours found that the application of lower loading densities (<= 235 kg/m^2) in the truck allowed pigs to have sufficient space to rest and arrive less fatigued at the slaughter plant (158).

Current industry guidance and customer standards require pigs be unloaded as soon as they reach the abattoirs (31). These requirements recognise that conditions on the truck may present a welfare challenge to animals, when the vehicle is stationary (for example, insufficient ventilation, smaller space allowance and unavailability of feed and water), with potential for pigs to experience thermal stress, fatigue, hunger and thirst, and restriction of movement. Delayed waiting times have been shown to exacerbate adverse welfare consequences, increasing pig mortality (159, 160), particularly if prior animal handling on farm and transport conditions have been poor (161-163). Maximum waiting times of 30 minutes have been recommended (159) as a sharp increase in mortality (more than twofold) has been seen after this time (164). The North American Animal Handling Guide (2021) (38), upon which the Australian Industry Standard (AMIC) (31) is based, requires animals to be unloaded within 60 minutes of arrival. Recent studies have examined the use of water sprays as a method of cooling pigs on stationary trucks (165-168). One study examined the effect of water misting with forced ventilation on internal vehicle ambient conditions, and behavioural and physiological response of market pigs (165). On arrival, trailers were kept stationary for 30 minutes before unloading. One trailer was positioned along a fan-misting bank (10 minutes of fan-assisted ventilation, followed by 10 minutes of ventilation and water misting and a final 10 minutes of fan-assisted ventilation), while the other trailer had no access to this cooling system. The researchers found that despite a variation in the efficiency of the cooling system between compartment location on the truck, this system appeared to be effective in improving the internal thermal environment and subsequently the thermal comfort of pigs. In another study (reported in three articles (166-168)) over a 12-week observation period, pigs were transported in either a conventional trailer (control) or one fitted with water sprinklers (that ran for 5 minutes before departure from the farm and again just prior to unloading). Trailers with sprinklers showed lower ($P=0.002$) increases in internal compartment temperature from loading to unloading, smaller ($P < 0.001$) decreases in humidity and no difference in ammonia levels. At an ambient temperature of > 23°C, there was no effect of sprinkling on pig behaviour on the trailer, but at ambient temperatures < 23 °C, more pigs stood on sprinkled trailers ($P < 0.05$). Sprinkling did not affect slips or falls during unloading. Once the pigs were unloaded into the lairage, sprinkled pigs spent more time lying ($P < 0.05$) and had fewer drinking bouts than controls ($P < 0.001$) regardless of ambient temperature. These data suggest that sprinkling pigs in a stationary vehicle when ambient temperature exceeds 23 °C has the potential to reduce the risk of thermal stress during short duration transport without detrimental effects on behaviour during unloading.

PG1.2: Unloading process

Pigs are usually unloaded from the truck using an angled ramp or a horizontal unloading bridge (159, 169). The ease of unloading can be influenced by the design of the unloading facilities. For example:

- Width of the ramp
- Ramp angle
- Flooring, for example, type and placement of foot battens
- Lateral protection, for example, railed or solid sides

Past research studies (pre-2001, therefore outside the scope of this review) have shown that a ramp slope >20° leads to an increase in heart rate, cortisol concentration, baulking behaviour and handling time, hence it is often quoted as a maximum slope in standards and codes of practice. More recent research, within the scope of this review, has re-examined the effects of ramp design and angle on a series of animal-based measures. A study on the impact of ramp angle on the total time to load and unload pigs and slips, falls, heart rate and vocalizations demonstrated that there is an effect of ramp angle on animal-based measures (170). The time required to load and unload and heart rate increased with increasing ramp angle, particularly during summer months when ambient temperature was higher. Another study examined the effects of ramp configuration (ramp slope, an initial step and angle of entrance to the ramp) on the behavioural and physiological responses of pigs. It found that the use of the steepest ramp slope (26 degrees) had the most detrimental effect on baulking and backing up behaviour of pigs ($P < 0.001$), and handling (touches, slaps, and pushes; $P < 0.05$ for all) during movement onto the ramp and on unloading time ($P < 0.01$). However, no differences in heart rate ($P < 0.05$) and ease of handling on the ramp ($P < 0.05$) were found between a 26 degree and 16 degree ramp slope, leading to a suggestion the length of the ramp may be one of the factors that makes unloading more difficult. They concluded that pigs appear more reluctant to move when a steep ramp, an initial step associated with a moderate slope, or a wide angle of entrance are used. For heavier weight pigs, such as sows and boars a reduction in the maximum ramp slope to 15 degrees has been recommended (60, 171).

A study of the impact of a novel environment on blood immune measures in pigs demonstrated that although pigs are not inherently stressed by alleys and ramps, exposure to a novel experience caused handling problems and a mild physiological stress response (172). The researchers found that handling ease and handling time were significantly improved ($P = 0.03$ and $P = 0.01$ respectively) when pigs were previously trained. The concept of previous training was also supported in a study of nursery pigs (173), where the researchers found that adding ramps to nursery pig housing improved the speed of loading pigs once they reached slaughter weight.

Compared to ramps, the use of hydraulic lifts or horizontal unloading platforms reduces handling stress, improves the ease of handling and shortens the time taken to unload (174).

PG1.3: Lairage facilities

PG1.3.1: Thermal comfort

Pigs have a very limited number of sweat glands, and therefore a limited capacity to lose heat by evaporation from the skin. Heat loss will generally occur through convection, conduction and radiation, influenced by the temperature difference between the skin of the pig and the environment. This means that pigs can be susceptible to heat stress, particularly if the ambient temperature is high and the environment during lairaging does not allow for adequate thermoregulatory behaviour. A combination of cold ambient temperatures and wind speed can also create a significant risk of wind chill for pigs, with older cull animals being particularly susceptible (38). A higher risk of death at a temperature range between 4 and 10°C compared to a range between 12° and 26°C has been reported (175). Indicators of inadequate thermal comfort can include observations of behaviours such as huddling, shivering and panting (160) and colour of the skin (176). In slaughter weight pigs, the thermoneutral zone varies between 15–28°C at a humidity between 40 and 80%. In pigs, lying behaviour is an important thermoregulatory tool which needs to be accommodated within the lairage environment. It has been reported (177) that at thermoneutral conditions, pigs take up to 80% of space allowance for lying and 20% for activity, which equates to 0.82 m^2 for a 110 kg pig. When the temperature increases (above 25°C), pigs tend to rest in lateral recumbency. At this higher temperature, the recommended space allowance is 1.10 m^2/100 kg live weight (178). There has been various work on heat mitigation strategies for pigs, with recommendations for showering and misting protocols covering characteristics such as density of spray, temperature of the water and duration of application . To optimise the cooling effect, it has been recommended that the water spray is heavy enough to penetrate the hair and wet the skin, rather than a fine mist which may just increase the humidity of the lairage (44). The use of a fine mist can be useful to control the concentration of dust and noxious gases (47), however, it is suggested that this is used in combination with effective ventilation to control the humidity.

PG1.3.2: Mixing pigs in the lairage

Mixed groups of pigs tend to fight in the lairage, with resulting skin blemishes and meat quality problems increasing with longer lairage periods (179). Group size was also found to be significant in one study, with pigs kept in large groups (30 animals) observed to spend more time standing ($P < 0.05$) and fighting ($P < 0.001$) than pigs kept in small groups (10 animals) (180). Interestingly, fighting was also found to be reduced in really large group of 200 pigs compared with smaller groups of 6-40 animals (181). A study of the provision of toys (rubber sticks and balls filled with maize) during transport and lairage to reduce fighting showed that pigs provided with toys had reduced prevalence of shoulder lesions and reduced glycolysis in muscle resulting in a lower lactate production and a slower pH decline. Blood sampling at sticking showed that pigs with the balls during transport and lairage also tended to have lower cortisol concentrations than pigs with the rubber toys (P=0·07) and the control group (P=0·08) (182).

PG1.3.3: Lairage noise

Noise levels above 100db have been found in pig lairages (180). Excessive lairage noise induces a fear response in pigs, as evidenced by increased heart rate, and greater blood lactate, CK and cortisol levels at slaughter (183). It is generally accepted that keeping the sound level lower than 85 dB in the lairage area appears to improve animal welfare and reduce the risk for of poor meat quality (184).

PG1.4: Holding duration

Under commercial transport and processing conditions in Beijing, a study showed that lairage time of 3 h led to a lower blood cortisol compared with pigs without rest, while longer lairage times of 8 h and 24 h resulted in a significant increase in pork toughness (185). The researchers concluded that three hours of lairage was appropriate to reduce pre-slaughter stress and obtain better meat quality for pigs transported for 4 h in winter. This corroborated findings of earlier research which found the optimal lairage time for pigs to be between 1 and 3 hours, using meat quality indicators (179), and 3 hours, using cortisol as an indicator (186).

A study used a measurement of oxytocin in the saliva samples of pigs as an assessment of emotional wellbeing after transport and lairage, where increased oxytocin was associated with positive emotions (187). Oxytocin was measured in 45 pigs before being transported to the abattoir, at the time of arrival and 4 hours after arrival to the abattoir. The results showed a decrease in free and protein-linked oxytocin concentrations 4 hours after transport compared with the time before transport (while cortisol showed an increase at 4 hours after transport compared with the time before transport). The researchers concluded that the transport and lairage (in the study conditions) produced a decrease in oxytocin in the saliva of pigs that could indicate a reduced emotional wellbeing.

PG1.5: Handling techniques

In a study on the effects of group size on handling, cardiovascular responses, time, and handling measures were collected (188). Group size significantly influenced heart rate, with increasing size associated with a linear increase ($P<0.05$) in heart rate. Once a threshold of seven pigs was reached, some leader pigs started to turn back making handling more difficult. It was concluded that although it is possible to move pigs in larger group sizes, this may have negative consequences for pig welfare and ease of handling.

One of the greatest problems in delivering animals to the point of slaughter is presented by the necessity for pigs to be handled in single file, such that they be moved individually into the stunning area.

A study to determine the impact of aversive handling on 91-day-old pigs (189), by use of the Human Approach Test, showed that pigs remember and avoid an aversive handler with whom they had contact three weeks previous, but do not avoid an unfamiliar handler. The authors concluded that piglets tested at 35 and 91 days of age demonstrate different avoidance responses to different handlers, determined by the quality of their previous interactions.

Pre-slaughter handling may also influence the stunning process. It has been linked to various responses to carbon dioxide stunning (190); where the researchers concluded that avoiding mixing pigs of different sexes in lairage and the implementation of good handling practices in the approach race may reduce the aversive response to CO_2.

PG1.5.1: Use of electric goads

Several standards recognise that the use of electric goads has negative animal welfare implications and prohibit or restrict their use during handling (31, 32). The use electric goads for moving pigs has been shown to increase heart rate, blood lactate, and salivary cortisol concentrations (191, 192), as well as the incidence of fatigued pigs (193). Furthermore, contrary to their intended purpose, electric goads have been shown to reduce the ease of handling (180). In a study of alternative handling tools, researchers found that electric goad use was associated with faster movement compared with paddles and compressed air prods, but was deemed to be more aversive (192). The paddle and compressed air prod decreased the number of slips/falls, overlaps, vocalizations, and lactate values compared with the electric goad, but also increased frequency of turn attempts and stops (resulting in at least 2-fold increase in loading time). The researchers concluded that additional research is necessary to identify methods to improve the loading efficiencies associated with alternative tools without compromising animal welfare. A further study (194) compared the efficiency and effects of flags, paddles and plastic boards and concluded that the plastic board and the flag were particularly effective as they appear to be a solid surface to the pig. A study of the relationship between beliefs, attitudes and observed behaviours of abattoir personnel in the pig industry found that a positive stockperson attitude was associated with use of an electric goad with the power turned off, while negative attitudes were associated with use of the goad with the power on (195).

PG2: Restraint

An individual pig may be restrained manually or mechanically in order to present its head to the operator for the purpose of correct application of the stunning method. Individual animal restraint is usually used when pigs are being stunned using electrical or mechanical methods. In small throughput abattoirs, individual or small groups of animals are held in small pens for stunning and shackling prior to bleeding (196). In larger throughput plants, mechanical restraint in the form of v-shaped conveyors or monorails are often used. As with all livestock, restraint can be a stressful process for pigs and consequently, restrained pigs should be stunned without delay. In the most modern controlled atmosphere stunning (CAS) systems, groups of pigs are stunned together to overcome some of the stresses associated with individual handling systems (24). The system is often operated with mechanical push gates that separate a large group of pigs into groups of five or six and move them into the gondola. The fact that pigs do not need to be individually restrained and can be stunned in a group is considered a major benefit in terms of animal welfare.

Section summary - Pre-slaughter period for pigs

Pigs have difficulties in descending slopes, with ramps steeper than 20 degrees resulting in greater stress (as indicated by animal-based measures such as heart rate, vocalization, backing up behaviour) and increased intervention by the handler. The use of electric goads has negative animal welfare implications. Alternative handling tools have been used successfully, however, often increase the time taken to unload. Pigs appear to benefit from a short lairage time (1-3 hours). Mixed groups of pigs tend to fight in the lairage, particularly during longer lairage periods. It may be possible to reduce fighting by avoiding mixing animals and providing enrichment during the lairage period. Restraint is a stressful process for pigs. Systems which allow the handling of pigs in groups rather than in single file are preferred.

PG3: Stunning

PG3.1: Controlled atmosphere stunning (CAS)

The use of controlled atmosphere stunning during pig processing has been extensively reviewed in recent publications (205, 213). The gas used commercially is carbon dioxide (CO_2) and the concept involves using the gas to displace atmospheric air in the system, such that a lethal hypercapnic (high CO_2) situation is generated and maintained until the animals are unconscious. Under commercial conditions, the commonly used method is by lowering individuals or small groups of pigs in a gondola into a pit that is pre-filled with a high concentration of more than 80% CO_2 (197).

An advantage with CAS is that pigs can be handled and stunned in small groups rather than separated and restrained individually (24, 198-200). Handling pigs in a group rather than using systems which require animals to move in single file has been shown to result in lower stress and improved movement through the system. Disadvantages of CAS, however, include the facts that the onset of unconsciousness is not immediate, and there is a period of time during which the animals perform behavioural responses indicative of aversion and

and distress (201-203). Furthermore, if animals are not killed in the system, there is a risk of return to consciousness if bleeding is not performed promptly and accurately (204).
A scientific opinion by the European Food Safety Authority (EFSA) (205) identified the following hazards to animal welfare when using carbon dioxide as a stunning method for pigs:

- Exposure to high CO_2 concentrations;
- Too short exposure time;
- Too low concentration of gas;
- Overloading of the gondola;
- Too low temperature of the gas.

The time needed to reach unconsciousness is shorter with increasing CO_2 concentration in the system. In a study of the induction of unconscious in CO2, time to loss of posture (considered to be a behavioural indicator of the onset of unconsciousness) was 38, 34, 25, 17, 22 and 15 seconds of exposure to CO_2 at the following concentration level 40, 50, 60, 70, 80 and 90% respectively (206). Similar results were found in a later study using a CO2 concentration of 80% and 90%, which resulted in unconsciousness at 21 and 15 seconds respectively (207).
Currently, no alternative method is available that offers the advantages of CO_2 but addresses the pain, fear and respiratory stress associated with the induction of unconsciousness (198, 205). It has been suggested that the only way to avoid the animal welfare issues associated with high concentrations of CO_2 in a CAS system is to use other gas mixtures (for example, inert gases) or mixtures of inert gases with low CO_2 concentrations (198, 200, 205). Alternative gas mixtures are not used commercially at present and have only been evaluated in experimental settings. There is further opportunity to explore the stunning of pigs using inert gases such as argon (or nitrogen), or gas mixtures containing up to 30% carbon dioxide in argon (or nitrogen).

PG3.2: Electrical stunning

In a commercial abattoir setting, electrical stunning is a commonly used method for stunning pigs prior to slaughter. The aim of electrical stunning is to pass an electrical current across the brain of the pig, resulting in an epileptic fit during which the pig is unconscious. Effective head-only electrical stunning is characterised by immediate collapse and onset of tonic seizures during exposure to the current (24, 208). To perform electrical stunning the animal needs to be individually handled and restrained (on a restrainer or in a pen) to allow electrodes to be applied to the head. Individual animal handling and restraint of this nature can be stressful (209).
Head-only electrical stunning does not kill the animal, but results in a recoverable state of unconsciousness (24). Therefore, this method must always be followed by bleeding (exsanguination). When electrical stunning is used, it is recommended that the stun to stick (bleeding) interval is kept as short as possible to reduce the risk of the pig recovering from the stun (a maximum of 15 seconds is recommended (24)).

Reviewed literature and guidance (15, 24, 210-212) concur that effective electrical systems require:

- Selection of equipment that ensures electrodes are an appropriate fit for the size of the animal;
- Accurate electrode placement to ensure good electrical contact (without pre-stun shocks);
- Sufficient restraint, such that contact can be maintained for the duration of the stun;
- Regular maintenance and calibration to ensure correct electrical parameters are being used.

The required electrical parameters for an effective head-only stun are defined in a number of guidance documents (15, 24, 68). The magnitude of the applied current should be 1.25-1.3 A at a low (50 Hz) frequency to meet the recommendations outlined in guidance. The implementation of constant current rather than constant voltage systems also improves animal welfare as they ensure that the minimum current required to stun the animal is consistently applied.

PG3.3: Mechanical stunning

Penetrative captive bolt devices are designed to fire a retractable steel bolt through the cranium and into the brain of the animal. Penetrative captive bolts used for pigs in a processing environment are normally powered by a blank cartridge, however, pneumatically-powered devices are also available. The desired outcome is for the impact of the bolt on the skull to result in concussion and the associated immediate loss of consciousness (24, 213). The structural damage to the brain may lead to rapid death of the animal, however some animals may not die immediately depending on the degree of damage to the brain (214). Consequently, bleeding (or other secondary procedure) should be used after an effective captive bolt stun.

Pigs can be difficult to stun with a penetrative captive bolt pistol due to the position and size of the brain relative to the head and the shape of the head (24, 215). Efficacy is thought to be directly impacted by the ability of the bolt to penetrate the skull. As pigs age, the thickness of the skull increases, the frontal sinuses expand, and some breeds may develop a bony ridge on the midline of the head (215). Shot position, and its influence on the areas of the brain that are damaged, will also determine the stunning outcome (24, 215). There has been little recent research on the use of penetrative captive bolt pistols in slaughter weight pigs, hence the frontal application site, as recommended by EFSA (24) and the Humane Slaughter Association (215), is still commonly used today. The position for placement of the device is described as the mid-line of the forehead, 1-2 cm above eye level. Alternative positions (behind the ear and temporal) have also been suggested for euthanasia, however researchers studying the basic tissue measurements and exposed cross-sectional brain area in cadaver heads (216) concluded that the frontal location may present less risk for the captive bolt euthanasia of swine at slaughter weight. Adult sows and boars often have a ridge of bone running down the centre of the forehead, which may prevent the bolt penetrating the brain. For this reason, the Humane Slaughter Association recommend that the shot position should be 3-4 cm above the eye level, with the muzzle of the captive-bolt slightly to one side of the ridge (215). It is also recommended that the highest grain cartridge is used for adult pigs. It is important, however, to refer to the manufacturers' instructions so that the correct cartridges are used for each model of captive bolt pistol.
Poor maintenance is a major cause of captive bolt device failure (215). Repeated firing of a captive bolt device for extended periods may also reduce effectiveness (217). The effect of the quality and consistency of blank cartridges on the stunning outcome has also been studied (218, 219), with large differences in cartridge performance being found. The researchers concluded that the variation in cartridge performance can be due to various factors such as fill volume and propellant function, and cartridge performance must therefore be a factor that is considered in the event of ineffective stunning.

PG3.4: Low Atmospheric Pressure Stunning (LAPS)

There is currently no published research on the use of LAPS for stunning adult pigs, however there is a significant body of relevant experience from investigations into the effects of LAPS for killing poultry, rats and piglets (220). A recent review (221) on the possible suitability of LAPS for pigs concluded that from research with humans and other animals it can be suggested that healthy, fasted pigs are unlikely to suffer from air hunger or from pain during LAPS. However, any pigs suffering from upper respiratory tract disease, tooth decay or excess gas in the alimentary canal may experience pain. To fulfil normal commercial throughput requirements, it is likely that an abattoir would need to install multiple decompression cylinders. The authors concluded that for most pigs LAPS is likely to be less stressful than current commercial slaughter methods.

PG4: Bleeding

PG4.1: Methodology

The principles of the bleeding process are discussed in GEN7. During processing, pigs are bled using a thoracic stick, severing the common brachiocephalic trunk which gives rise to the carotid arteries. A knife is inserted into the ventral aspect of the neck just in front of the sternum, towards the thoracic inlet. Studies on the effectiveness of pig slaughtering procedures recommend that for effective sticking, the blade of the knife needs to be long enough to reach the appropriate blood vessels and the size of the incision should be large enough to allow profuse bleeding (222, 223). In a study comparing short and long sticking incisions (222), a long incision of 11.2 cm (S.D.=3.6) resulted in more rapid bleeding that a short incision of 4.5 cm (S.D.=2). Recommending a long sticking incision to promote good welfare may however meet with opposition due to concerns over contamination of the carcase during the scalding process.

PG4.2: Time to brain death

It is important that death occurs during unconsciousness, therefore it is necessary to understand the period of unconsciousness produced by the stunning method and the time to brain death as a consequence of blood loss. Foundational research in this area (224) demonstrated that after chest sticking of pigs, the time between the first appearance of blood from the sticking wound and to loss of brain responsiveness (based on reduction in Visual Evoked Potential - VEP) was found to range between 14 and 23 sec (18, SD: ±3 sec) whereas time to loss of VEP after cardiac arrest ranged from 17 to 22 sec (19, SD: ±2 sec). The same authors found the development of an isoelectric electrocorticogram (ECoG) to range between 22 and 30 sec after the chest stick. In a project to investigate the causes of inadequate sticking encountered during a survey of pig abattoirs (222), it was found that this period can be affected by variations in commercial practice and the slaughter process may be prolonged. The main causes of ineffective sticking were found to be related to operator error as a result of high throughput, tiredness, insufficient instructions, animal position, inadequate knives as well as convulsions caused by effective stunning currents resulting in handling problems. The authors concluded that by ensuring a 'relatively long sticking wound by a thoracic cut' and addressing operator training and training should lead to improvements in the process.

Section summary

With effective sticking, the time to brain death in pigs is relatively short (range between 14 and 23 sec (18, SD: ±3 sec) when using an assessment of the loss of evoked potentials. Thereby, the subtraction of the time to loss of evoked potentials following accurate sticking from the minimum duration of apparent unconsciousness produced by an accurate stun head-only electrical stun, the recommended stun-to-stick interval can be calculated as 15 s. The size of the sticking wound makes a significant contribution to the effectiveness of the process and time to brain death.

PG5: Animal welfare assessment

PG5.1: Assessment of unloading and handling

The welfare of pigs may be compromised from the time of loading on the farm until the arrival at the abattoir, therefore assessment of the welfare state of pigs at arrival is important. Animal-based assessment methods have been used in audit protocols developed in the US (26, 38, 61, 225) and also integrated into the WelfareQuality framework™ (25, 160, 169, 226, 227). In the cited articles, the suggested measures for the assessment of the unloading and handling process include measures of general fear (reluctance to move and turning back), thermoregulation (panting and shivering), slipping and falling, lameness, sickness (defined as those animals unable to walk) and deaths of animals. A study on the use of animal-based measures to assess handling (227), concluded that assessment of lameness was shown to be reliable (good inter-observer reliability) when assessed from the unloading bay to lairage, whereas slipping and falling should be scored in the unloading area of the abattoir. The authors suggested an assessment should include a maximum of two measures of fear on the same animals at the unloading area, with the most reliable parameters being turning back and reluctance to move.

In pigs, negative emotions may be manifested by freezing, defecation, urination, attempts to escape, acute vocalizations, lowered tails, and ears pressed backward or in movement (228). Vocalisation scoring has also been used to assess the impact of handling methods, equipment and facilities, however, in pigs this can be quite complicated. It is difficult to count individual vocalisations when a group of pigs is being handled, therefore vocalisation scoring is often restricted to individual pigs held in restraint, stun box, or group stunning pen (38). The nature of the vocalisation is also important in pigs, with squeals counted rather than grunts (38). A study aimed to develop a system to monitor and record levels of stress calls (screams and squeals) in pigs, which could be employed in environments of breeding, transportation and slaughter (229). The team used sound analysis detect the stress vocalisations of pigs on-farm, employing a system which was insensitive to environmental noise, human speech and pig vocalisations other than screams. The detection quality in commercial systems was found to correlate well with that of human experts.

Facial expressions of animals are a means of manifesting emotions in several species and can be used to predict aggression or pain (230). A recent article has provided the possible use of facial expressions in pigs to indicate wellbeing (231). It was concluded that, to-date, the only proposed facial expression scale for pigs is based on an assessment of painful events (such as castration and tail docking) in piglets (piglet grimace scale). However, this could serve as the basis for developing a facial expression to evaluate other animal emotions, such as the expression of fear during pre-slaughter handling.

PG5.2: Assessment of stunning and slaughter

Indicators of the presence of consciousness during bleeding, leading to pain and fear in pigs are presented in an EFSA report (21). They are summarised as the presence of: Muscle tone; breathing; corneal/palpebral reflex; blinking and posture (failure to collapse or attempts to regain posture). In addition, it is recommended that death be recognised by the absence of movements, cessation of bleeding and dilated pupils. A recent study investigated the benefits of hot-water spraying as a diagnostic test to verify the absence of signs of life in pigs after CAS and before scalding (232). Animals being conveyed from sticking toward further processing were sprayed with hot water on the muzzle, head and front legs, and behavioural indicators of life were assessed. The stick-to-spray interval range was 143 to 258 s. The researchers concluded that use of a hot water spray was a promising test for signs of life, where spontaneous movements and sustained mouth opening in particular were regarded as indicators of compromised animal welfare.

Sheep

SH1: Pre-slaughter handling

SH1.1: Unloading process

The physical unloading process may be assumed to be stressful, but in the case of sheep it appears that there are no significant effects on physiological indicators of stress such as plasma cortisol concentration or body temperature (233).

SH1.2: Lairage facilities

The noise levels in lairages are often greater than sheep would be accustomed to on farm (47), and noises such as barking dogs, banging gates, whistle and rattles can have a substantial effect on animal movement (234). Space allowances in lairages can become reduced during periods of high turn-off, and this can affect the ability of sheep to rest prior to slaughter. Lying and drinking behaviours of sheep held in a commercial abattoir lairage in Australia for 24 hours were compared at space allowances of 0.3, 0.45, 0.6 and 1.0 m^2 per animal (235). There was a significant positive linear relationship between space allowed and lying behaviour (i.e. the more space allowed, the more time sheep spent lying and the greater number of sheep observed to be lying), but there was no impact on drinking behaviour. In all groups, 20% of observed sheep failed to drink during the 24 h lairage period. The authors concluded that the optimal space allowance to ensure adequate hydration for sheep may be greater than 1.0 m^2. It is also possible that in sheep, failure to drink water while in lairage may be unrelated to space allowance, and more related to other factors such as unfamiliarity of the surroundings or unfamiliarity with the intrinsic parameters of the water provided.

SH1.3: Holding duration

In terms of rest in lairage prior to slaughter, there is a general assumption that better welfare and meat quality outcomes in red meat species are associated with longer rest periods. This is certainly the case in sheep, but only up to a certain point. Each published article accessed considered different measures and different lairaging periods, in sheep of differing ages and transported for differing durations, so it is difficult to draw firm conclusions on optimal lairage duration, and there may well be interactions between transport duration and environmental conditions that are as yet unelicited.

Researchers (236) studied sheep (average 6 months of age) that had been transported for 8 hours, and then lairaged for 0, 2, 6, 12, 24 or 48 h with ad libitum water, measuring a variety of stress-related plasma constituents and white blood cell differential counts; and meat quality attributes (carcase weight, dressing percentage and ultimate pH (pHu)), They found that sheep in the 48 h group had reduced carcase weight, reduced dressing percentage, higher pHu, higher neutrophil count, packed cell volume, total protein, and blood urea nitrogen as compared with other groups, with the 24 h group showing similar increased parameters, but not significantly different from either the 48 h group or the 12h and under groups. These findings suggest a degree of dehydration and physiological stress in the sheep lairaged for 24 h or more, leading the authors to recommend a 6-12 h lairage period for sheep post transportation.

Similarly, lairage durations of 30 min, 2.5, 5 and 15 h were compared in ewe lambs (8-9 months of age) that had been transported for 2 h, again measuring a variety of plasma constituents and meat quality attributes (237). The researchers sampled blood on arrival at the abattoir and at exsanguination, finding that although sampling time did significantly affect plasma cortisol, glucose, lactate dehydrogenase and creatine kinase, lairage duration did not have an overall significant effect on these parameters. Cortisol was reduced from arrival to exsanguination in all lairage groups, indicating recovery from the acute stress of transportation and handling. Glucose increased in the 30 min group (which may be a response to the activity around unloading and handling), was not significantly altered in the 2.5 and 5 h groups, and reduced in the 15 h group (suggesting a degree of negative energy balance). Lactate dehydrogenase and creatine kinase were significantly increased in the 30 min and 2.5 h groups (which may be a response to the activity around unloading and handling), not significantly altered in the 5h group, and lactate dehydrogenase was reduced in the 15 h group (which may be indicative of recovery from the activity around transportation, unloading and handling). The 15 h lairage duration also led to a significantly reduced pHu as compared to the other groups, (suggesting a degree of physiological recovery) but there were no significant differences in the other meat quality parameters. These authors concluded that 15 h in lairage allowed the lambs to fully recover from the stress of transportation, but in light of the limited effect on meat quality parameters, and regulatory restrictions on lairage duration in Europe (where the study was conducted), they suggested that 2.5 or 5 h lairage duration could be recommended.

Concentrations of a variety of plasma constituents, pHu and meat quality attributes of lambs (approximately 15 weeks of age) were compared in two groups: slaughtered directly on arrival or lairaged for 12 h (overnight) prior to slaughter (238). The transportation was 1 h. The lairaged group had significantly lower plasma cortisol, lactate, and glucose concentrations, but significantly greater creatine kinase activity and non-esterified fatty acid concentrations. The authors concluded that the 12 h lairage period reduced the stress in lambs at the time of slaughter as compared to non-lairaged lambs. Similarly, other researchers (239) also concluded that a 12 h lairage period allowed lambs to recover from a 3 h transportation period, after comparing meat quality characteristics of lambs that were slaughtered without transportation, slaughtered after 3 h transportation, or slaughtered after 3 h transportation with 12 h lairage. The meat quality characteristics of the lairaged group and the untransported group were not significantly different, whereas the transported but not lairaged group showed significant detrimental effects on pHu and shear force. A lairage period of 12 h or more was seen to be more beneficial (240), having compared 0, 12 and 24 h lairage after what was described as 'short distance transportation', although the duration of transportation was not reported. The 0 lairage group produced tougher meat and higher pHu than the lairaged groups. The conclusions of this study are limited by the fact that no intermediate lairage period between 0 and 12 h was studied.

Interestingly, another study (241) found that an 18 h overnight lairage period after 75 minutes transport did not significantly affect plasma cortisol, lactate dehydrogenase, glucose or creatine kinase (measured on arrival and at exsanguination) but there was evidence of dehydration (packed cell volume and total protein increased) in 19-20-week -old lambs. However, meat quality attributes (tenderness, colour, water holding capacity and cooking loss) of the 18h lairage group were improved as compared to a group that were lairaged for 30 minutes, leading the authors to recommend lairage durations of greater than 30 minutes. In an Australian study (242), lambs from 9 different consignments were split into three subgroups per consignment: slaughtered on arrival; lairaged for 1 day or lairaged for 2 days. The lambs were supplied under the normal commercial supply chain for the abattoir, so travel times ranged from 30 minutes to 9 h. Travel times were balanced across lairage groups. Muscle glycogen changed according to lairage duration, but the degree and direction of change was not consistent across consignment – some increased muscle glycogen, others decreased muscle glycogen and in others there were no significant changes by lairage time. Differences in pHu were only significant in two consignments. Transport duration had a significant effect on the glycogen concentration of semimembranosus muscle (lambs that had traveled the longest had higher muscle glycogen), and on the pHu of the semitendinosus muscle (lambs that had travelled longer had higher pHu). In another Australian study (243), lambs were held in a paddock at the abattoir for 1 week to recover from transportation, then walked to the lairage and held for either 1 or 2 days prior to slaughter. The pHu of the 2-day lairage group was significantly higher than the 1-day group, suggesting an element of chronic fatigue and muscle glycogen depletion during the extended lairage period.

The impacts of lairage may be in part due to the novel environment, but the fasting imposed also has an effect. In a research situation, where lambs were fasted for 0, 12, 24 or 48 h prior to slaughter (without transportation), lambs in the 24 and 48 h groups had significantly higher plasma cortisol and triglyceride concentrations than the 0 h group, with 12 h intermediate (244). Creatine kinase and blood urea nitrogen concentrations were significantly higher in the 24 h than the 0 and 12 h groups, with levels in the 48 h group intermediate, suggesting a physiological re-balancing, perhaps associated with water intake.

Investigating shorter lairage durations, in (245) it was identified that cortisol concentrations were significantly reduced in lambs (approximately 21 weeks of age, transported for 90 minutes) lairaged for 3 h as compared to those lairaged for 1 h, suggesting that 3 h lairage had allowed some degree of recovery from transport. In contrast, in another study (246) no significant difference was found between groups of 13-week-old lambs lairaged for 0, 2 and 4h after a 30 minute transportation in terms of plasma glucose, creatine kinase, lactate dehydrogenase, alanine aminotransferase and meat quality attributes, leading those authors to conclude that lambs could be slaughtered with minimum lairage after a short transport period. A positive linear association between lairage duration and pHu in mutton sheep was identified in a study at a small abattoir in South Africa (247), suggesting that increasing durations of lairage resulted in increased fatigue. However, the authors did not report on the range of lairage durations studied, nor on the conditions of lairage and handling at the abattoir. In a similar study by the same authors (248), sheep were held for 1.5 -2 hours prior to slaughter, and again a significant positive association with pHu was identified.

Under Australian conditions, many sheep will be transported for 8 hours or more to slaughter (e.g. from WA to the eastern states) (249), so for these animals, provision of a lairage period of the order of 6-12 hours appears to be indicated from the above data. However, interpretation of the currently available data is challenging: most studies either consider curfew with or without transport, or lairage duration against a standard transport period, and there are no controlled studies examining the interactions between the three aspects (curfew, transport and lairage), on which to base recommendations.

SH1.4: Handling techniques

The behaviour of stockpersons and their interactions with animals is known to have an effect on the animals' physiological stress response and behaviour (6). Recent research has focused on the implications of stockperson behaviour in abattoirs.

The attitude of stockpersons influences their behaviour. When stockpersons felt that they had limited control over their actions or felt that they were under time pressure they were more likely to perform inappropriate handling behaviours (5). Similarly, when stockpersons believed that the facilities made animals more difficult to handle, they were more likely to whistle and make noise; while those that believe that goad use is not stressful, and animals must be aroused or 'stirred-up' to make them move better were more likely to make more noise and use the goad more often.

Use of dogs in Australian sheep abattoirs is common practice, reducing the need for direct intervention between the handler and the sheep. However, the presence of a dog is stressful to sheep. During sheep handling, at two different abattoirs, plasma cortisol was significantly correlated with increased dog use, which in turn was associated with decreased whistles, and decreased touches or pushes from stockpersons (6). In a later study, the duration of fast locomotion of the stockperson and lifting or pulling of lambs was associated with increased physiological stress (measured using cortisol, glucose and lactate concentrations), as were the duration of dog behaviours such as barking or lunging at the animals (250). It has been recommended that dogs are not to be used in close quarters with sheep, and should be limited to pastures, large pens and other open areas where animals have room to move away (12).

The dual objectives of efficiently moving animals through the lairage to slaughter while also ensuring good welfare presents a significant challenge to processors: maximising one can lead to negative consequences for the other, so a balance is required. A pilot study (251) on 'Optimal Flow' investigated the movement of sheep though a linear series of five pens into a curved 'bugle' pen, a triangular forcing pen and finally a linear race at a commercial slaughterhouse in Australia. The objective of the study was to identify handler and dog behaviours that correlate with stress-related behaviours of sheep and stalling or baulking. A total of 3253 mixed age meat sheep, five stockpersons and seven kelpie-type dogs were observed by video recording over a 3-day period. Sheep behaviour was observed as groups, followed through the entire series of pens and race, while dogs and personnel were observed as individuals. Detailed ethograms of the behavioural events or states recorded were provided. Distress behaviours in sheep were correlated with the presence of a dog, and with slow movement through the system. High sheep density was associated with a higher flow rate through the system, but also greater distress behaviour, and greater incidences of forcing or pressure by dogs. Forcing or pressure from dogs reduced the rate of sheep movement. The authors identified that Optimal Flow occurred in the system evaluated when sheep were at lower densities. In this study, stocking densities were described as categories, and the model suggested that Optimal Flow occurred when sheep density was category 3 "Loose" or less. Category 3 was defined as "Sheep occupation of space is such that there is approximately one sheep-body-width in two directions surrounding the majority of the sheep in the group", while Category 4 "Moderate" was defined as "Less than one sheep-body-width in at least one direction surrounding the majority of sheep in the group, but sufficient empty space around the sheep to allow sideways or forward movements that create the space for a single sheep in the group to pass between two other sheep in the group". Further work is required to determine if these densities would lead to Optimal Flow in all abattoirs.

(6, 12, 250)

SH2: Restraint

Restraint, particularly when the animal is isolated from its conspecifics, can be extremely stressful to sheep (252). V-restrainer conveyors, which are designed to allow sheep to maintain visual, tactile and audio contact with one another, allay the effects of isolation. Sheep are reported to be calmer and show less distress or avoidance behaviours when presented to the stunning location using a v-restrainer than presented manually (253, 254). However, in some jurisdictions, notably the UK, there is a requirement of animals to be 'individually and mechanically restrained', particularly in the case of non-stun slaughter. This has been interpreted to meant that sheep must be individually loaded into the v-restrainer, with the restrainer being emptied prior to loading the subsequent animal; which defeats the intention of the v-restrainer design principles of using the sheep's natural following instinct to assist smooth flow onto the restrainer. A study evaluating the impact of such a practice found that sheep that were individually loaded onto the restrainer struggled more and had increased plasma lactate and cortisol concentrations when compared to sheep that were allowed to flow smoothly and sequentially onto the v-restrainer (255).

SH3: Stunning

SH3.1: Mechanical stunning

The physical brain damage resulting from use of both penetrating and non-penetrating captive bolts (Schermer brand, No 17 cartridge) on anaesthetised 4- 5-week-old lambs has been evaluated (256). The lambs were maintained under anaesthesia and ventilated for 2 hours post stunning. The brain damage produced by both instruments indicated that either instrument would effectively stun lambs. In a second study, using 2-year-old sheep, similar brain damage was observed (257). In the latter study, four of ten lambs were not ventilated post stunning, and all of these died within 10 minutes of stunning. The six ventilated lambs survived until euthanased two hours after stunning.

SH3.2: Electrical stunning

Recent research into electrical stunning of sheep has focused on optimising stun parameters to ensure an effective stun without risking the development of blood splash (small haemorrhages throughout the muscle tissue), a carcase quality problem that may be associated with over-stunning of smaller animals (258). Thus, a number of studies have explored the minimum current application required to achieve an effective stun. Researchers (259) used head-only electrical stunning on lambs in the weight range 10-15 kg. 0.6 A applied for 10.5 s produced an effective stun in only 34% of animals, 0.8 A was effective in 64% of animals, 1.0 A was effective in 86%, and 1.25 A for 10.5 s was effective in 92% of lambs processed in a commercial slaughterhouse (n = 50 in each group). These same researchers also studied the effect of duration of application, applying 1.25 A for either 3 s (n = 60) or 14 s (n = 75). The short duration application increased the likelihood of ineffective stunning, and the presence of reflexes that are indicative of consciousness or returning consciousness. There was no significant difference in the incidence of blood splash in the carcases studied. In small lambs and goat kids, 7-16 kg, 50 Hz currents of 0.3, 0.5 and 0.7 A applied for 3 s were compared to the 1.0 A specification in EU legislation (260). Effective stunning, as indicated by epileptiform EEG and behavioural responses was achieved in all 360 lambs studied and there were no significant differences in duration of stun, leading the authors to recommend that currents of 0.3 A for 3 s were suitable for small lambs and goat kids.

Another approach has been to look at factors affecting effective stunning and duration of stun. One study compared (261) placement of scissor-type stunning tongs (frontal – between the eyes and ears on either side of the head; and caudal, behind the ears on either side of the head), whether the tongs were wet or dry, and whether the sheep were woolled or shorn. The stun used 250V, 50Hz applied for 0.2 s, the lambs were 18-22 kg bodyweight and electroencephalogram (EEG) data were used to assess stun quality ad duration. Stun effectiveness (proportion of animals showing epileptiform behaviours and high amplitude EEG) was significantly greater in the frontal than caudal application positions; significantly greater when tongs were wetted than dry, and significantly greater when the sheep were shorn than woolled. This was also true of the duration of epileptiform EEG, but in terms of time to returning reflexes, the data were less conclusive.

SH3.3: Gas stunning

There has been recent interest in using carbon dioxide (CO_2) inhalation for stunning small (up to 25 kg bodyweight) lambs. When comparing electrical stunning (110 V, 50 Hz, 5 s) against dip-lift immersion in 90% CO_2 for 90 s (262), there were significantly lower plasma adrenaline concentrations in gas-stunned light (~25 kg) lambs, and significantly lower cortisol concentrations in gas stunned suckling (~12.5 kg) lambs. There were no other significant differences in cortisol or catecholamine concentrations, leading the authors to conclude that CO_2 inhalation could be a suitable alternative to electrical stunning of lambs. However, aversive behaviour, head-shaking, sneezing and breathlessness has been reported in lambs that were progressively immersed into 90% CO_2 (263).

In another study (264), electrical stunning (110 V 50 Hz, 5 s) was compared with two different CO_2 concentrations (90% or 80%) for two different exposure times (90 s or 80 s) in a gondola dip-lift system when stunning suckling lambs (approximately 30 days of age, liveweight 12.5-13 kg). In this study, 100% of electrically stunned lambs were fully unconscious but not dead after the procedure; 90% CO_2 at either exposure time resulted in all lambs being either unconscious or dead; 80% CO_2 for 90 s resulted in all lambs being either unconscious or dead; but 80% CO_2 for 60 s resulted in 28.5% of lambs remaining semi-conscious based on presence of movement and reflexes. There were no significant differences in cortisol or catecholamine concentrations between groups, while haemoglobin and leucocyte counts were greater in the 80% CO_2:60 s group than either 90% CO_2 groups and creatinine was greater in the electrical stunning group than in the 80% CO_2:90s group. The authors concluded that 90% CO_2 would be more appropriate to use in terms of effective stunning and physiological indicators of stress. An almost identical study in older lambs (265) (aged 70 days and 25 kg liveweight) found that 80% CO_2 resulted in 50 or 70% (at 90 and 60 s exposure respectively) of lambs being semiconscious, while cortisol and catecholamine concentrations were significantly higher in the 80% CO_2:90 s group than all other groups. Electrical stunning resulted in significantly greater plasma creatine kinase, lactate dehydrogenase, sodium and potassium than the CO_2 stunning groups. Those authors concluded that 90% CO_2:60 s would be the most appropriate stunning method for these lambs. A parallel publication by the same authors (266) reported on meat quality parameters associated with the study, finding detrimental tenderness and pHu effects associated with the 80% CO2 groups.

SH3.4: Application of microwave energy - DTS: Diathermic Syncope®

The electromagnetic energy system described in section CA3.3: Application of microwave energy - DTS: Diathermic Syncope® has been tested on four anaesthetised sheep. The required increase in brain temperature to induce insensibility was achieved, and EEG traces indicated that an epileptiform state (incompatible with consciousness) occurred (267).

SH4: Bleeding

There is some suggestion that handling of sheep can influence bleeding rate. In two studies in South Africa (254, 268), avoidance behaviour of sheep seemed to correlate with bleeding rate, although the data are confounded by gender, breed and age. The groups exhibiting higher avoidance behaviour scores also had longer bleeding times (measured from beginning of flow to dripping).

'False aneurysm' or 'carotid ballooning' leads to reduced rate of bleeding and can lead to the risk of an animal that has been reversibly stunned regaining consciousness during exsanguination. In a study of 411 lambs slaughtered in the UK, 6.1% of arteries inspected showed signs of carotid ballooning. In no sheep were both arteries affected (88). The recommended stun to stick interval (when reversible stunning methods are used) in the OIE Terrestrial Animal Health Code (15) is 20 seconds.

SH5: Animal welfare assessment

There is interest in using infra-red thermography (IRT) of the eye to assess negative emotions, e.g. fear, in animals. In sheep the temperature of the lacrimal caruncle has been shown to increase as a result of restraint (269), but the technique has not yet been validated in a commercial processing system.

Goats

GO1: Pre-slaughter handling

GO1.1: Lairage

Goats are social animals and isolation is stressful. A study to investigate the effect of isolation on stress responses in goats, placed goats into 15 minute isolation without visual contact with other goats; 15 minute isolation with visual contact; and control group-housed animals (270). Animals were blood-sampled before and after treatment, assaying a range of parameters. Only cortisol was affected by the treatment – goats in the isolated groups had significantly greater cortisol concentrations than goats in the control group. The difference in cortisol concentration between isolation without visual contact and isolation with visual contact was not significant.

The potential benefit of providing 0.97 kg crude glycerin to goats lairaged for 12 hours, compared to those provided water alone was studied (271). The plasma cortisol concentration was lower in the goats provided crude glycerin, suggesting that these animals were less stressed than the ones that were lairaged with water alone. There were no effects on meat quality parameters or behaviour during lairage. A similar study also demonstrated that provision of crude glycerin to goat kids during lairage reduced stress , but the effect was stronger when the goats had also received crude glycerin during feedlotting (272).

GO2: Stunning

GO2.1: Mechanical stunning

A study using Magnetic Resonance Imaging and Computed Tomography compared brain damage resulting from penetrating or non-penetrating captive bolt applied behind the horns in 1-5 year-old goats (273). Both methods caused similar brain damage, leading to the authors concluding that the non-penetrating device may be an acceptable method of euthanasia for goats. Recommendations of correct shooting position are: for polled goats, midline, in the middle of the forehead, just above the level of the eyes, aiming down along the angle of the neck, and for horned goats midline, just behind the bony ridge where the horns protrude, aiming toward the back of the chin (274) OR at the cross-over point of two imaginary lines drawn between the base of the horn and the opposite eye (275).

GO3.2: Electrical stunning

Scientific evaluation of electrical stunning in goats in the period 2000-2021 has tended to focus on the comparison of electrical stunning against slaughter without prior stunning. Details of these studies are presented in section Gen6.4: Slaughter without stunning. Electrical stun parameters tend to be inferred from sheep, although some specific recommendations have been made, e.g. 1.0 A for 8 s (Dayen 2001, cited in (17)). A study that compared low frequency head only (LFHO, 1.0 A, 50 Hz for 3.0); low frequency head-to-back (LFHB, 1.0 A, 50 Hz for 3.0) and high frequency head-to-back electrical stunning (HFHB, 1.0 A, 850 Hz for 3.0) was conducted using minimally anaesthetised goats (n = 8 in each group) (79). Electroencephalogram (EEG) and some plasma biomarkers were evaluated. After electrical stunning, there was an increase in EEG activity across all frequencies, but after sticking the activity was significantly reduced as compared with baseline, suggesting that they were insensible to pain during and after the neck cut. There were no significant differences between stunning groups in terms of EEG activity or plasma biomarkers (glucose, lactate, total protein, urea, creatine kinase, calcium, adrenaline, noradrenaline and lactate dehydrogenase). Behaviourally, goats that were stunned using LFBO performed significantly less severe clonic (kicking/convulsive) activity after stunning than goats stunned using LFHB or HFHB.

Blood splash (ecchymosis) is a problem encountered with goat kids, so some research into reduced current application has been carried out. A study involving 120 kid goats (7 kg bodyweight), stunned either head-only or head-to-back, using 50 Hz currents of 0.3, 0.5 and 0.7 A applied for 3 s were compared to the 1.0 A specification in EU legislation (260). Effective stunning, as indicated by epileptiform EEG and behavioural responses was achieved in all animals studied and there were no significant differences in duration of stun, leading the authors to recommend that currents of 0.3 A for 3 s were suitable for goat kids.

From EFSA (17):

"Captive bolt and electrical stunning methods are used in goats. It is assumed that they are effective by analogy to research findings with sheep and other species.

When mechanical stunning is used it must be followed by bleeding.

In the absence of any evidence that use of carbon dioxide to stun goats is humane, high concentrations of carbon dioxide should not be used.

Research should be undertaken to underpin the use of captive bolt and electrical stunning methods with goats."

GO4: Bleeding

Goats are usually slaughtered using a transverse neck cut. There is little recent research on time to loss of consciousness, but a dissertation study measured corneal reflex in 10 goats after slaughtering without prior stunning (276). Here, loss of corneal reflex occurred after 3 – 7 s of exsanguination. Conversely, during the DIALREL process (a project aiming to address issues relating to religious slaughter by encouraging dialogue between stakeholders), corneal reflexes were elicited up to 120 s after sticking (7).

GO5: Animal welfare assessment

No specific information for the assessment of goats at the time of slaughter was found, with most reviews inferring that information pertaining to sheep is relevant (See Section GEN3.1).

Horses and donkeys

HS1: Pre-slaughter handling

HS1.1: Unloading ramps

Researchers investigated training methodologies for loading horse prior to transport (277). Although the data presented are not relevant to the current review, they described the facility used. The horse were held in an outdoor area which was “connected to a load lane (14.5m long), leading to a concrete ramp (6m long, 8% of slope) and the trailer (trailer ramp: 1.5m long, 10% of slope)”. Similarly, research on horse behaviour during transport (278), although not relevant to the current review, described unloading facilities at a number of slaughterhouses in Argentina and Uruguay: “The slope of the loading docks was on average 17.4° (±3.6)°, which equals 31.5% (±7.0)%. Seven out of 21 loading docks had a slope steeper than 20.0° (36.4%) and the slope of all but one loading dock was steeper than 10.0° (17.6%). The length of the loading dock (measured on the surface of the loading dock) was 4.01 (±0.90) m and the height was 1.18 (±0.17) m”. In Australia, the current MCoP prescribes that slope of ramps should be no greater than 20° (1 in 3), while the Australian Standard AS 5340:2020 (279) requires the slope of fixed ramps to be no greater than 20°, but adjustable ramps are permitted to be up to 25°.

A scoring system, based on WelfareQuality™, to assess horses arriving at the slaughterhouse after long-distance transportation (280) has been developed. The research tested the assessment methodology on 51 shipments of horses. Some of the findings have relevance to the current review. When the unloading ramp had rubber matting, horses were far less likely to slip than when there was no rubber matting ($P = 0.002$). As ramp slope increased, the risk of horses falling increased, and this correlation was statistically significant ($R = 0.39$; $P = 0.005$). In that study, 58% of ramps assessed had slope greater than 20°, but a full data range was not provided, so it is difficult to draw conclusions as to optimal ramp slope from the available data. However, the findings suggest that ramps of greater than 20° slope may contribute to decreased welfare in horses, the use of adjustable ramps (permitted to be up to 25° under AS 5340:2020) may not be suitable for horses. More detailed research into the use of adjustable ramps in horse processing in Australia is warranted.

HS1.2: Unloading process

During unloading, researchers (280) identified a relationship between the animals’ willingness to move and handling techniques. Appropriate handling was associated with greater willingness to move ($W = 389$; $P = 0.003$), while reluctance to move was associated with high prevalence of inappropriate handling behaviours such as slapping/hitting the animal ($W = 119.5$; $P = 0.002$), or ‘moving excitedly’ by the handler ($W = 92$; $P = 0.04$). Furthermore, when handlers were making loud noises during unloading, a greater proportion of animals tended to show fast movement instead of calm movement ($W = 108.5$; $P = 0.05$).

HS1.3: Lairage facilities

There was no specific research identified on optimal lairage conditions or the effects of the lairage period on horses. See Section Gen4.2: Lairage for general principles.

HS1.4: Holding duration

Researchers measured blood parameters in 1438 equids (mules, donkeys, rodeo horses, cull horses, foals and ponies) at saleyards in Mexico (281). Blood samples were taken one hour after arrival at the saleyard, and five hours after arrival, and tested for carbon dioxide saturation (pCO_2), oxygen saturation (pO_2), acidity (pH), glucose, lactate, bicarbonate, haematocrit (packed cell volume, PCV), sodium (Na), calcium (Ca) and potassium (K). They found that even at the first sampling point, all the horses were showing physiological signs of dehydration (despite ad libitum water being available), and fatigue, with compensatory metabolic changes. Mules and donkeys were most severely affected. Based on this research a maximum of one hour holding time could be recommended.

HS1.5: Handling techniques

See handling during unloading (HS1.2). Researchers have attempted to reduce the stress in slaughter horses by applying a mentholated ointment to the nostrils of horses while in the lairage prior to slaughter (282). In treated horses, there was significantly lower post-stun catecholamine concentration as compared to untreated horses ($P < 0.05$), but no differences in cortisol or β-endorphin. They concluded that blocking the olfactory response could modulate the stress response to slaughter. However, the horses in that study were well handled, stabled heavy horses specifically bred and reared for meat production. The operator safety aspect of attempting this procedure in poorly handled feral or excitable young 'hot-blooded' animals would be a significant challenge in the Australian industry.

HS2: Restraint

There was no specific research identified on restraint methods or the effects of restraint on the welfare of horses. See Section GEN5: Restraint for general principles.

HS3: Stunning

The efficacy of stunning was evaluated (283) (based on indicators of insensibility) and brain pathology of 46 horses and ponies stunned using a .22 calibre rifle with hollow point rounds. Researchers concluded that free-bullet shooting is an effective means for horse slaughter, as all were immediately rendered insensible and all had damage to the brain structures.

In an abattoir study in Chile (284), 333 horses were observed being slaughtered using different stunning equipment. One used a cartridge-driven non-penetrative captive bolt, achieving 76.6% first stun efficacy, with 19.1% showing signs of returning consciousness and 84.4% bled within 1 minute of stunning. 33.3% of heads examined had the shot position within 2 cm of ideal. In a second abattoir which used a pneumatic penetrative captive bolt, 78.2% of horses were stunned on first shot, 17.8% showed signs of return to consciousness and only 5% were bled within 1 minute of stunning. 11.5% of shots had landed within 2 cm of ideal. In the third abattoir, electrical stunning was used, using a single-electrode application. Only 2.9% were stunned on first application, 3.1% showed signs of returning consciousness and 66.3% were bled within 1 minute of stunning. The authors concluded that the main objectives of stunning (no unnecessary pain during slaughter) were not being achieved. No similar contemporary data were found pertaining to the Australian industry, and in the absence of such data there is a risk that the results presented from the Chilean study are assumed to apply to the Australian industry context, whether or not they are indeed relevant. There should be further research into the overall welfare implications of the use of captive-bolt as a stunning method in horses.

Plasma cortisol, haematological, biochemical and antioxidant enzyme concentrations were tracked in 24 warmblood horses from lairage (60 minutes prior to slaughter), through to the point of slaughter (immediately prior to penetrative captive-bolt stunning) and exsanguination (285). The study was carried out in Slovenia, and the description of the pre-slaughter handling indicated that the horses were familiar with handling. This is corroborated in the finding that there were no differences in the concentrations of any measure taken in the pre-slaughter phase, although the baseline (taken one hour prior to slaughter, and the horses had been lairaged for varying periods of 2-5 hours prior to baseline sampling) may itself be elevated as a result of transportation and lairage, and the one-hour interval between baseline and slaughter may not have been sufficient to result in changes in concentrations. However, between immediate pre-stunning and exsanguination, concentrations of many of the variables (lactate; glucose; potassium; aspartate aminotransferase; creatine kinase and most of the other biochemical and haematological variables) were significantly ($P < 0.05$) increased. This is likely to be due to the stunning process itself activating the sympathetic nervous system.

In a similar study (286), blood constituents (plasma lactate, glucose, creatine kinase (CK) cortisol and haematocrit (PCV)) were tracked in 21 cull race horses through transport, lairage, and slaughter (penetrative captive bolt) in Chile. Transport duration was 45-85 minutes, and time in lairage was 18-21 hours. Blood samples were collected 1 h before loading; immediately after loading; on arrival at the slaughterhouse, before unloading; in the lairage pen, immediately after unloading; after lairage, in the lairage pen; in the stunning box before stunning; after stunning, during exsanguination. Horses were held in the stunning box for 1-22 minutes. 85.7% were stunned on first shot, and 57.2% showed signs of recovery. The stun-stick interval was most commonly between 1 and 2 min (although greater than 2 min in 23.8% and up to 4 min in 9.5% of horses). There was a significant ($P < 0.05$) increase in lactate, cortisol and PCV during transport. Plasma glucose and cortisol dropped during lairage, and all measures spiked at exsanguination. The authors concluded that the slaughter process was a source of stress, the observations of poor stunning practice aligning with spikes in physiological variables. However, the physiological data were not further subdivided into groupings relating to 'effective first stun', 'mis-stun' and 'observed return to consciousness', so it is unclear as to the relative influence of improper stunning on the data collected, and the conclusion that the slaughter process itself is a source of stress is not necessarily fully supported. It may also be misconstrued, as the process of stunning itself leads to activation of the sympathetic nervous system and changes in the physiological measures, which from a physiological point of view is 'stress', but in common parlance, 'stress' is often interpreted as being a psychological process with associated physiological change.

HS4: Bleeding

There was no specific research identified on bleeding methods and associated welfare outcomes for horses. See Section for GEN7: Slaughter general principles.

HS5: Animal welfare assessment

A list of potential welfare indicators in 2642 horses arriving at the slaughterhouse were investigated in the study (287). Cluster analysis indicated that lameness, nasal discharge, ocular discharge and skin wounds could collectively assign animals to four separate welfare categories (Good, Average, Poor and Very Poor).
A further study on horses in the abattoir (288) identified that carcass bruising formed distinct patterns which, although being influenced by body condition of the animals, could be attributed to specific issues during the pre-slaughter phase. Cluster analysis identified four distinct patterns which they defined as: Severe and Concentrated Damage Pattern (SCDP); Rear Limb Non-Severe Pattern (RLNP); Thoracic Wall Non-Severe Pattern (TWNP) and Disperse Non-Severe Damage Pattern (DNDP). The SCDP pattern was the most severe and was related to inter-individual conflict, e.g. kicking and biting; RLNP was related to loss of balance during transport; TWNP was related to orientation in the vehicle and DNDP was related to impact against infrastructure. A non-invasive method of infra-red thermography (IRT) was used by researchers (289) to identify subclinical injury (e.g. bruising) prior to slaughter. In 93 horses slaughtered in Canada, bruising was identified in 54% of carcases at the inspection point. The IRT method had 42% sensitivity (i.e. 42% of bruises were correctly identified) and 79% specificity (i.e. 79% of non-bruised areas were correctly identified). The authors concluded that the approach used was not good enough, and refinements to either the technology and/or the procedure would be required to bring it to a commercially viable option.
Animal-based measures of welfare were also proposed in a scoring system, based on WelfareQuality™, to assess horses arriving at the slaughterhouse after long-distance transportation (280). The assessment methodology was tested on 51 shipments of horses, with the conclusion that the proposed assessment protocol was repeatable and practical under commercial conditions, and would provide a means of benchmarking and monitoring welfare applicable across industry. Transport stress in horses has also been investigated (290) using a variety of behavioural, clinical, haematological and biochemical measures . The study concluded that the behavioural and clinical measures (heart rate and rectal temperature) were more sensitive than the haematological and biochemical measures utilised in differentiating between stress levels in horses.

Deer

D1: Pre-slaughter handling

D1.1: Handling

EFSA (17) recommend “specialised deer abattoirs, equipped with specific lairaging and restraining/handling facilities”, but interestingly there is no information on what that resembles in practice.

Killing methods for three groups of fallow deer on a game reserve were studied: the first group had been mustered to a paddock or corral on the day prior to shooting; the second were handled from the corral into a race and restraint box then stunned using a captive bolt pistol; and the third were shot while roaming freely on the range (291). Animals that were shot while free-ranging had lower meat pH, and highest muscle glycogen than the two mustered groups, leading the authors to recommend this as the optimal killing method. The recommendation was based on meat quality parameters, but the parameters used are strongly influenced by stress and exertion, so it could also be inferred that shooting under free-ranging conditions would be optimal in terms of welfare.

A similar study in farmed red deer (292) also indicated that shooting on the farm was preferable to mustering, transport and handling in terms of stress measures (cortisol, progesterone, glucose, lactate, albumin, creatine kinase, lactate dehydrogenase, aspartate aminotransferase and packed cell volume) ($p < 0.05$), but there were not significant differences in meat quality parameters (muscle glycogen, pH, colour, shear force and sensory panel evaluation).

D1.2: Lairage facilities

Deer appear to have a fear of other species (293, 294),so specialist deer facilities would be recommended over processing in multi-species facilities. Welfare of deer in lairage can be improved by reducing light levels and increased pen size. A study of 8 groups of 10 yearling deer indicated that when the deer were held in dark conditions (0-1 lux), they dispersed more evenly across the pen, spent more time exploring, and were less vigilant and performed less escape activity in the presence of a handler than when they were kept in light (200 lux) conditions (295). In terms of space allowance, when groups of 10 2-year-old deer were held in pens that were 4 m x 5 m, the animals spent significantly less ($P < 0.05$) time pacing and head tossing compared to when held in pens that were 2.5 m x 4 m (296). It has been recommended that space allowances for deer are a minimum of 2–6 m^2 per animal and animals should be held in groups of 6 or more (297).

When designing handling facilities, the ability of deer to jump must be taken into consideration - most species will easily jump 2–3 m. Goddard recommended that gates should be a minimum of 3.5 m wide and raceways should be fully enclosed to reduce visually apparent opportunities for escape, and that lead-in sections be 5-10 m wide to minimise bunching and congestions. Deer prefer to move in small groups, staying 2-3 abreast, and move more easily through curved raceways (298).

Deer should be slaughtered soon after arrival at the abattoir. When red deer were kept for 3, 6 or 18 hours in the lairage, it appeared that 6 hours allowed the deer to recover from transportation (pHu was lowest in the 6 h group), and behaviour returned to on-farm baselines after 8-10 hours. However, in the 18 h group, there appeared to be an increase in aggressive or agonistic behaviours, leading the authors to conclude that deer should not be lairaged for long periods (299).

D2: Restraint

A report by the Farm Animal Welfare Council (FAWC) (3) recommended that deer should be restrained in a drop-floor crate. In an additional guidance document (297), a V-shaped restrainer, with padded sides, between 2 and 3 m long, in which the floor may be dropped was recommended as a suitable method for the close restraint of deer.

D3: Stunning

The most common method of stunning method for domesticated deer in an abattoir environment is the use of a penetrating captive bolt pistol, but head-only low-voltage electrical stunning and firearms (free projectile) are also used. No published articles on the effective mechanical stunning parameters were identified, however, the bolt velocity and hence the magnitude of the charge and type of equipment may vary with different species and type of deer. The use of a firearm (free projectile) is an effective method for killing deer, however, its use is usually confined to deer killed in the field. When head-only electrical stunning is used, minimum currents of 1.0 A and 1.3 A have been recommended for fallow and red deer respectively (300-302).

D4: Bleeding

Deer may be exsanguinated using the thoracic sticking method or the transverse neck cut. The thoracic sticking method results in the fastest rate of blood loss, based on weight of blood collected over the first 10 s of exsanguination (303).

D5: Animal welfare assessment

There was no specific research identified on animal welfare assessment methodology for deer during slaughter. Most articles which use animal-based assessments of stunning and slaughter for deer tend to infer that the information for other livestock species, e.g., cattle, is applicable. See Section GEN3: Animal welfare monitoring for underlying principles of animal welfare assessment.

Camelids

CM1: Pre-slaughter handling

CM1.1: Lairage

Evidence of stress during lairage has been identified in camels. In one study that compared lairage durations of 12-16 hrs against lairage durations of 16-20 hours, camels in the longer lairage group had lower cortisol, neutrophil:lymphocyte ratio, thyroxine and triiodothyronine than the camels in the shorter lairage group, but in both groups these parameters were elevated compared to immediately after unloading (304). This suggests that the lairage period itself was stressful, but as camels were held for longer, there was some physiological adaptation.

In another study, 28 camels were walked 2 km to lairage and were clinically examined (rectal temperature, heart rate and respiratory rate) and blood sampled for haematology evaluation on arrival, after 3 h lairage and after 10 h lairage (305). The clinical parameters were reduced by lairage relative to arrival, while red blood cell count, haemoglobin level and lymphocyte count increased at the 10 h lairage timepoint. Those authors concluded that a 3 h lairage period was optimal to limit the stress in camels.

CM1.2: Handling techniques

The attitude of handlers influences the ease of moving/handling alpacas, in a similar manner to that identified for other livestock species (4). In one study of 184 alpaca handlers (306), where handlers had a generally negative attitude moving and leading alpacas was more difficult ($p < 0.05$), but where handlers had received attitudinal training, moving and leading alpacas was easier ($p < 0.05$). Handlers that talked to the alpacas found moving and leading easier ($p < 0.05$), and behavioural problems in alpacas were associated with negative handler attitudes ($p < 0.05$). These points were supported by the findings of a more detailed study by the same authors involving 116 alpacas on 20 alpaca farms (307).

A study in Australia compared overnight lairage against 7-days' lairage in groups of male alpacas that had been transported 4 h to the abattoir, and then divided into the two lairage groups (308). Blood samples were not taken on arrival, but at slaughter, blood was collected for cortisol determination and meat samples were collected for quality analysis. There was no difference between groups in terms of cortisol concentration, suggesting that both lairage periods had allowed the alpacas to recover from the acute stress of transportation, but meat quality attributes (tenderness, drip loss and muscle glycogen content) were adversely affected by the 7-day lairage period.

CM2: Restraint

CM2.1:

A solid-sided squeeze restraint for alpacas or llamas, while a crate or stocks similar to those used for horses, but adjusted in dimensions, would be utilised for camels (309).

The method of restraint can be stressful to alpacas. In a study on restraint for shearing, alpacas that were allowed to remain standing performed less vocalisation, kicking or struggling, and had greater heart rate variability than animals that were cast on the ground or on a table (310). In the hour after shearing, the animals that had remained standing spent more time lying, feeding or ruminating than the cast animals. Thus, the authors concluded that standing restraint is to be recommended for shearing. No data are available to support or refute this recommendation in the context of slaughter.

CM3: Stunning

CM3.1: Mechanical stunning

One study examined the brains of 96 alpacas stunned using a penetrative captive bolt gun and found that the crown (top of head) position led to the greatest damage to the brain structures that are related to consciousness (311). Behavioural observations during the study indicated that 90.2% of the alpacas were effectively stunned on first shot, those that were not stunned effectively were those in which the instrument was incorrectly applied, missing the brain completely.

Although the puntilla stab is not considered a humane slaughter method in Australia, it is still used in a number of overseas countries. A study in Bolivia examined 20 llamas slaughtered using the puntilla stab followed by neck sticking (312). In 9 animals (45%), multiple stabs were required to pierce the foramen ovale. Two of the animals attempted to stand after the first stab, only one animal lost the corneal reflex and all animals showed rhythmic breathing after puntilla.

CM3.2: Electrical stunning

There was no specific research identified on electrical stunning methods and the associated welfare outcomes for camelids. In an article on the processing of camels (313), electrical stunning is covered as a topic area, however the reference material only covers cattle and sheep.

CM4: Bleeding

There was no specific research identified on bleeding methods and associated welfare outcomes for camelids. See Section for GEN7: Slaughter general principles.

CM5: Animal welfare assessment

There was no specific research identified on animal welfare assessment methodology for camelids during handling and slaughter. Most articles which use animal-based assessments of stunning and slaughter for camelids tend to infer that the information for other livestock species, e.g., cattle, is applicable. See Section GEN3: Animal welfare monitoring for underlying principles of animal welfare assessment.

Rabbits and guinea pigs

RA1: Pre-slaughter handling

RA1.1: Holding facilities

Rabbits are transported and held in containers prior to processing. It is recommended that the crates used to hold rabbits should have solid floors to prevent soiling of animals in lower tiers (314). Other features of the holding environment that would impact on the thermal comfort of rabbits include the height of the container stacks, the space between the container and the environmental conditions (for example, temperature, humidity and airflow) inside the lairage area (314).

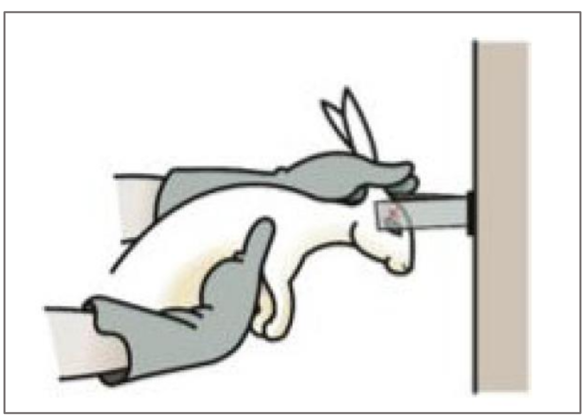
RA1.2: handling techniques

Rabbits should be removed from the containers individually by holding and lifting by the neck (scruff) by one hand, with or without support of the body with the other hand. The ears or back legs should not be used to lift and carry rabbits (315).

RA2: Restraint

Rabbits are mainly stunned by using head-only electrical or captive bolt stunning, both of which require individual animal restraint. Restraint applied for head-only electrical stunning aims to ensure the electrodes span the brain (316, 317). Adequate restraint is also crucial to ensure proper placement of the bolt during penetrative captive bolt stunning (17, 316). Restraining for head-only usually involves holding the rabbit with one hand supporting its belly, while the other hand guides the head into the stunning tongs or electrodes by holding the ears (Figure 02), notwithstanding the fact that holding by the ears alone is prohibited in the EU (46). It should be noted that restraint may differ for overhead electrodes.

Figure 02: Restraint for head-only electrical stunning (Source: European Commission, 2018 - (317))



RA3: Stunning

RA3.1: Mechanical stunning

In commercial abattoirs, the main method used for rabbits is electrical stunning although penetrative captive bolts have been used. It is recommended that the captive bolt device is placed against the rabbit's head on the midline and at the intersection of lines drawn from the outside edge of the eye to the base of the opposite ear (316). If applied in the correct position, a penetrative captive bolt gun with a 6 mm diameter and 27 mm deep bolt is considered to be effective (316) and the depth of penetration should be deep enough to impart forces to the brain stem. Penetrative captive bolt stunning is therefore deemed to be effective in rabbits, though only causing death of the animal in some instances (17). It is therefore recommended that it is followed by bleeding to ensure death.

Guinea pigs are slaughtered using cervical neck dislocation, captive bolt, electrical stunning or carbon dioxide CO_2 inhalation. In a study comparing methods (318), captive bolt appeared to be the most humane and effective, leading to 9 of 10 animals (90%) effectively stunned, while only 3% of 60 animals slaughtered using cervical neck dislocation were deemed successfully stunned, 97% having maintained reflexes or behavioural responses. 94% of 83 animals electrically stunned (head only, 140 mA) were successfully stunned, while CO_2 inhalation (flow rate of 20-30% chamber volume per minute) was associated with respiratory effort (heaving and gasping) during the 2-3 minute induction period.

The use of a penetrating spring-loaded captive bolt for euthanasia of guinea pigs has also been studied (319). In that study, 12 of 12 guinea pigs were immediately rendered unconscious using the instrument, with loss of reflexes and loss of rhythmic breathing immediately after application.

RA3.2: Electrical stunning

Head-only electrical stunning is used commercially for rabbits, however, there has been little scientific research to establish the optimum stunning parameters. In an EFSA report (17), it was recommended that a minimum current of 400mA should be achieved until such time that further research has been completed. Electrical stunning of rabbits is generally carried out using a handheld or wall-mounted V-shaped spiked electrode. The rabbit is supported by the operative in one hand while using their other hand to guide the rabbits head between the fixed electrodes.

RA3.3: Controlled atmosphere stunning

The use of a CAS system for rabbits would remove the need to handle and stun individual animals (17), however, there has been little research on the effects of exposure to gas on the welfare of rabbits, in relation to the stress of induction or the parameters required to stun animals effectively. Exposure of conscious rabbits to more than 70% CO_2 causes painful stimulation of the nasal mucosa and aversive reactions. Behavioural indicators of aversion include vocalisations, rubbing the nose with the forelimbs, headshaking and gasping (320, 321). A more recent study examined the behavioural and physiologic responses of rabbits when undergoing CAS, with a chamber filled at a gradual rate vs. fast rate (322). The researchers observed minimal differences in behavioural responses between fill rates with no clear signs of distress. Time to loss of consciousness was faster with the fast fill rate. Rabbits progressed from a loss of balance to loss of posture to loss of righting reflex (unable to right itself from a position on its side). Loss of righting reflex was used as the primary variable to judge loss of sensibility and occurred within seconds (9 ± 1 s) of loss of posture. Open

mouth breathing occurred in 65% of trials, however it was observed after the rabbit had lost its righting reflex. Vocalizations occurred inconsistently for both fill rates but was a behaviour displayed by more rabbits in the fast-fill group.
There is no clear research on the concentrations of CO_2 that elicit minimum aversion in rabbits, whilst producing an effective stun. The use of controlled atmosphere systems to stun rabbits is not permitted in Europe (316) and is not covered by the OIE Terrestrial Animal Health Code (15).

RA3.4: Low atmosphere pressure stunning (LAPS)

Low Atmosphere Pressure Stunning (LAPS) has not been researched for use in rabbits.

RA4: Bleeding

The Australian standard for the hygienic production of wild game meat for human consumption AS4464:2007 contains basic references to killing of game in the field but does not contain specific detail of the slaughter process. In Europe it is a requirement to sever both the carotid arteries following stunning (EC No 1099/2009). Under commercial abattoir conditions, this is usually performed by making a ventral incision on the surface of the neck of the rabbit behind the mandibles. During the processing of rabbits, the stun-to-stick interval is usually less than 10 s (316) with a survey carried out in a Northern Italy recording an average stun-to-stick interval of 5.5 seconds (S.D. 0.88) (323).

RA5: Animal welfare assessment

Welfare consequences associated with the slaughter process and relevant animal-based measures (indicators) are described in an EFSA report on the stunning and slaughter methods for rabbits (316), with animal-based measures suggested for each stage of the process from unloading to slaughter.

Poultry

The definition of poultry for the purpose of this review is aligned with the current MCoP, that being: chickens (meat and layer), turkeys, ducks, geese, guinea fowl, pheasants, partridges, quail and pigeons.

PO1: Pre-slaughter handling

PO1.1: Arrival and unloading

When poultry arrive at the processing establishment their transport containers are either kept in lairage on the truck or unloaded and moved to designated areas. Placing poultry into a well-ventilated location, with protection from direct sunlight and inclement weather can prevent negative welfare consequences (324). Commercial practices usually involve holding birds in lairage for several hours depending upon the throughput rate and other operational factors. The total time between catching and slaughter (i.e., a combination of transport duration and holding time) has an impact on mortality, where increasing time in crates above 4 hours was shown to increase the number of broiler chickens 'dead-on-arrival' (DOA) (325). Commercial practices vary from unloading poultry upon arrival and moving them straight to the point of stunning without lairage, to holding them in lairage for some hours. The approach depends upon the throughput rate of the processing plant and other operational factors, such as shift pattern. In Australia, most poultry are held in the lairage for at least a few hours prior to slaughter, with the holding time often determined by the compliance and industry requirements around total time without feed and water, for example, as outlined in the Land Transport Standards and Guidelines (42).

PO1.2: Lairage

PO1.2.1: Noise and lighting

There has been no research on the effects of noise in the lairage on poultry welfare, however, intensities of sound measured in cattle and pig lairages has been found to often exceed 85dB (47). There is also growing evidence in meat chickens and laying hens of aversion to noises around this intensity, that affects both behaviour and productivity (326, 327).

Lighting is often kept at a low level in poultry lairages as it is thought to reduce bird activity in the crates. As cited in the Farmed Bird Welfare Science Review (328), dim lighting (<5 lux) has been associated with reduced activity compared to brighter lighting (20-320 lux), however, it has been reported in one study that activity in 6 week old broilers was similar under dim and bright light intensities (5 or 100 lux, adjusted to fowl perceived illuminance). More recently a study aimed to settle the debate by examining the preference of broiler chickens for light intensity. A choice system was developed to allow determination of the preferences of broiler chickens for light intensity (329). This system had three light proof pens each with feeders or waterers but different light intensities. The study provided evidence that the preference of meat chickens is for 20 lux (compared to 5 and 10 lux) light intensity for activities such as feeding. The authors argued that the requirements for resting and feeding are more complex than a simple minimum light intensity, but suggested that light intensity may be reduced in areas where birds are resting.

PO1.2.2: Thermal comfort

The thermoneutral zone for meat chickens and laying hens (within which the bird is thought to be comfortable and does not need to expend any effort in to keep warm or lose heat) is thought to be around 20-25 °C. The thermal requirements of other poultry species are less well established. Thermal stress is influenced by bird weight, fat cover, feather cover and by air movement, radiation (e.g., sunlight) and conduction (heat loss through contact with a surface). Birds may adjust their own body temperature by behavioural thermoregulation - i.e., by altering their posture, in particular spreading out their wings and standing when hot and huddling together when cold - and by seeking out a warmer or cooler place in their environment, which should provide the choice and freedom to move. Heat stress and mortality can be increased in crates which prevent birds adopting these postures, for example, when higher stocking densities are used (330). As birds are held in their transport crates during the lairage period, efficacy of ventilation in the lairage is important. Ambient temperatures above 17^{o}C, during transport and subsequent lairage, have been shown to increase mortality in meat chickens (330). The microclimate inside the crate is likely to be higher temperature and humidity than the surrounding area, with the majority of DOAs observed in a 'thermal core' where ventilation is deemed to be sub-optimal (331, 332). This has implications for the stacking pattern of crates in the lairage to ensure adequate airflow around the birds. If the lairage environment can be climatically controlled, it may be opportunity to reduce the thermal load of poultry after transport in hot conditions. A Brazilian study (333) of broiler chickens transported in the spring and summer months, found a lairage period of 3–4 h in suitable conditions was sufficient to reduce thermal load and pre-slaughter mortality.

PO1.3: Handling

PO1.3.1: Handling and fear

Handling itself can raise stress levels in poultry (334) and poultry are generally fearful of human contact (335). On-farm, fearfulness can potentially be reduced through appropriate habituation (336),however this may not be as significant in a processing environment, where birds are exposed to many novel stimuli. There has been little work on the impact of fear of humans and stockmanship on birds during the processing phase.

PO1.3.2: Handling chickens and turkeys

Physical handling and restraint can elevate underlying fear levels in broilers, and this effect can persist after the event. In the Farmed Bird Welfare Science Review, it was reported that meat chickens which experienced 'pleasant' human contact (gentle stroking) showed reduced fear reactions to transportation compared to a control treatment (no contact); broilers subjected to unpleasant handling (inverted swinging and aversive noise) had similar fear responses to the control group. Methodologies for assessing how comfortable animals are with people have been developed and applied, and human–animal relation tests are the tools most commonly included in welfare assessment protocols (e.g., avoidance distance test in the Welfare Quality® assessment protocol for poultry (337). Since the publication of the Farmed Birds Welfare Science Review there has been little additional research handling as it affects chicken and turkey welfare, although the impact of animal welfare training for poultry handlers has been studied (338), showing that welfare measures such as flapping at shackling, pre-stun shocks, stun parameters and effective neck cut showed significant improvement post training.

PO1.3.3: Handling other poultry species

There are very few studies on the handling of other poultry species, published after 2017. In an article determining animal-based measures for geese (339), researchers used a handling test to assess the human-animal relationship. They found 100% inter-observer reliability when evaluating the attitudes of stockpeople and the reactions of geese to humans. These interactions are associated with animal welfare parameters such as levels of fear, stress responses, and productivity. In quail, there are some on-farm studies examining the impact of handling during husbandry procedures which have provided an insight into how quail respond to individual handling and restraint. Handling quail for weighing was found to elicit a significant adreno-cortico response when compared to control birds (340). Guinea fowl are slightly smaller and flightier than chickens. However, unlike the recommendations for chickens, the Humane Slaughter Association recommend that guinea fowl are handled and carried supporting the body (341). There were no scientific references that covered the lairage or holding of guinea fowl before slaughter. Pheasants are typically quite flighty birds, therefore practical handling techniques usually involve holding both legs, with the head and body tucked under the handler's arm. Catching can involve the use of a net placed over the bird to enable the handler to pick up the bird whilst supporting the body (341).

PO1.4: Humane killing sick and injured birds

The use of cervical dislocation is covered by both the Australian Standard and Model Code of Practice; which stipulate that 'poultry shall be rendered unconscious by dislocation of the head' (AS 4465:2007) and 'cervical dislocation' can be used for birds that are not stunned effectively by the primary method. Much of the research in the past focused on neck dislocation in meat chickens, however, there have been a number of studies post-2017 on the use of neck dislocation in turkeys (342, 343). Cervical dislocation in poultry can be divided into two types of technique. The first involves stretching the neck resulting in extensive damage to the spinal cord and rupture of major blood vessels (341, 344), whilst the second involves crushing the neck with pliers. The effects of neck stretching are very different from those associated with neck crushing. Neck stretching methods consistently broke the spinal cord, whereas crushing methods sometimes fail to do so. Consequently, neck dislocation with pliers is not a recommended technique and is prohibited in some certification scheme standards (33, 345). When manual cervical dislocation was compared to mechanically assisted (using a Koechner device) cervical dislocation (346), it was found that reflexes were lost sooner, brain death occurred faster and internal damage was greater after application of manual cervical dislocation compared to the mechanical methods. Although the researchers did not estimate the time to loss of consciousness, they recommended manual cervical dislocation over the use of the Koechner device. There are differences in opinion regarding the effectiveness of cervical dislocation and its ability to result in immediate brain dysfunction, with several recent studies concluding that manual cervical dislocation results in rapid loss of brain function and onset of brain death (Brainstem reflexes: (344, 346-348); musculoskeletal movements: (346)), whilst other studies (predominantly pre-2017, but summarised in an EFSA review(24)) have shown that cervical dislocation, either neck crushing or stretching, may not lead to immediate brain death in turkeys or chickens.

In more recent studies it has been inferred that successful manual cervical dislocation is dependent on the ability of the operator, influenced by operator fatigue, bird size and bird type (344, 347). In the EU, manual cervical dislocation may only be used for birds less than 3kg (46).

Mechanical stunning, during which a non-penetrating percussive blow is applied to the head of the bird, has been used for killing poultry and may offer an alternative solution to neck dislocation. The commercially available devices use concussive force to interrupt the functioning of neurons, disrupting sensory processing and causing traumatic brain injury that leads to unconsciousness and death. To achieve an effective stun when using mechanical stunning equipment, the bolt diameter, velocity, airline pressure (or cartridge strength), application position and angle are critical parameters (24). The effectiveness of commercially available devices has been investigated in chickens (349), turkeys (342, 350, 351) and ducks (352). In the study on 35-day old Pekin ducks (352), researchers found that a commercially available device (Zephyr-EXL) and an experimental crossbow immediately abolished eye reflexes and therefore were deemed to meet the criteria for a successful euthanasia method.

PO1.5: Shackling

When a shackle line is used, birds are either removed individually from transport crates by the shackling operatives, or are tipped from the crates into a hopper, and onto a series of conveyor belts leading to a shackling carousel. Shackling requires birds to be inverted and causes compression of the birds' legs which can result in pain and distress (328). Much of the research around shackling is focused on meat chickens, however, the risk to welfare is likely to be similar for all birds.

The pain experienced by birds during this shackling can be exacerbated further by rough handling, design of the shackle, the time period between shackling and stunning, and additional features of the line that cause the birds to flap. Birds that flap after shackling are likely to experience further pain compared to those that are calm and settled (328).

In the past, the time between shackling and entering the waterbath was up to 3 minutes for chickens and 6 minutes for turkeys (24) but faster times are now being achieved (around 1 minute for both species). The suspension time should always be as short as possible. For example, EFSA (24) and the OIE (15) recommended a maximum shackling time of one minute but EFSA reported 12 or 20 seconds may be sufficient time for chickens and turkeys respectively, to settle on a shackle line.

The design of individual shackles is also very important as they have the potential to compress the tissues of the shank, including the periosteum and the tarsometatarsal bone, if they are not appropriate for the size of the bird. Poultry processing plants must use shackles that have the correct size (gauge) aperture for the birds' shanks. This is problematic in plants that process different poultry types (e.g., end of lay hens and meat chickens) and sizes on the same shackle line. If the shackle gauge is too small, it can damage the leg of the bird or prevent the leg being placed in the correct position.

The process of shackling has been shown to be stressful to chickens, with studies summarised in the Farmed Bird Welfare Science Review (2017) (328) and more recent studies (353). A maximum time that birds are shackled for has been managed through industry and customer standards in an attempt to reduce the impact the process on the birds, however, research into the effect of shackling time on the welfare of the bird is limited. The RSPCA Approved Farming Scheme Standard limits total shackling time from hang-on to entry into the waterbath to 60 seconds for meat chickens (33) and layer hens (354) and 90 seconds for turkeys (345). A recent study (355) has examined the effects of shackling duration on plasma concentrations of corticosterone and heterophil to lymphocyte ratios in slaughter weight turkeys. It was found that transport increased corticosterone, however, there was no further increase in plasma concentration of corticosterone after shackling or with increasing duration of shackling. There was also a decrease in H : L ratios following shackling. The researchers concluded that the data supports the hypothesis that an extended duration of shackling in turkeys should not be viewed as unduly stressful, consistent with an evidence-based approach, however, they also suggested other possible explanations for the lack of an effect of shackling on plasma concentrations of corticosterone, such as possible down-regulation of the HPA-axis due to other stressors. In a similar study of broiler chickens (356), which evaluated the effect of shackling on plasma levels of adrenaline, noradrenaline and corticosterone, researchers found that shackling for less than 30 seconds did not elicit a physiological stress response.

PO2: Stunning

PO2.1: Electrical stunning waterbath stunning

PO2.1.1: Waterbath design

Stunning is achieved by the passage of an electrical current from the electrode in the waterbath through the bird to the shackle line. Waterbath stunning systems can be different in their design, utilising either a:

- Deep bath of water (covering an electrode)
- Shallow bath of water (covering an electrode)
- Wet electrode (wet plate system)

Electrical waterbath stunners are single phase, driven by either an alternating voltage waveform (AC) or a pulsed direct voltage waveform (pDC), or multiphase. In multiphase systems, two treatments of differing waveforms and current amplitudes are applied consecutively. The effects of multi-phase systems on brain activity are largely scientifically unknown.

Electrical waterbath systems used for meat chickens are usually designed for around 10 to 25 birds to be in contact with the water/electrode at the same time (depending on the length of the waterbath), though are usually shorter for the other poultry species. In this type of multiple bird waterbath stunning system, all the birds passing through the waterbath will be exposed to a constant voltage. This means that the flow of electrical current through the bird is dependent on the resistance of each bird to current flow, such that birds with a low resistance will receive more current than a bird with high resistance. The contact between the legs and shackle significantly influences the resistance of the bird to current flow. Factors that can increase resistance at the leg/shackle interface include:

- Shackling on top of a severed foot
- Shackling by one leg
- Poor shackle position
- Dry shackles
- Presence of scale on the shackle surface
- Thin legs (for example, female birds compared to males)
- Keratinised skin on the legs (e.g., older birds).
- Body weight (influencing downward force in the shackle and leg size) (357)

The Farmed Bird Welfare Science Review (328) summarises the welfare risks and compromises of the electrical waterbath as follows:

1) *The birds are removed from their transport crates and handled at speed*
2) *The birds are inverted and suspended from a shackle*
3) *The shackle is likely to put pressure on the legs causing pain*
4) *The birds are at risk from painful pre-stun electric shocks as they approach the water-bath*
5) *Wing flapping due to these stresses can result in broken wings*
6) *Agitated birds may occasionally struggle and avoid being electrically stunned*

7) *The electric current delivered to each bird varies and so some birds may not be adequately stunned.*

These welfare compromises will be common amongst all types of bird stunned in an electrical waterbath and can be reduced by good staff training, well-designed and maintained equipment and correct parameter selection, however they cannot be completely avoided. The summary of electrical waterbath stunning provided by the Farmed Bird Welfare Science Review (328) states:
Electrical water-bath stunning is the most widely used and most widely researched slaughter technique for poultry. It can result in immediate and long-lasting unconsciousness which, when followed rapidly by reliable bleeding, results in death without recovery. The approach however includes inherent welfare compromises and the system requires careful setup and constant management to protect bird welfare.

PO2.1.2: Pre-stun shocks

In each electrical waterbath systems, birds are exposed to the possibility of receiving a pre-stun shock before they are stunned, although the risk can be greater with a reduced depth of immersion (for example, with the wet plate system). The RSPCA Approved Farming Scheme Standard for Meat Chickens (33) requires that "equipment, calibration, and procedures for electrical stunning must have as their primary purpose that birds do not receive pre-stun shocks." In a wet plate system, the bird's head is required to make direct contact with the electrode as the shackle line moves through the waterbath. The ability to maintain good electrical contact in this type of system is difficult, as any variation in bird size can cause them to lose contact with the metal plate (358).
Other factors that increase the prevalence of pre-stun shocks at the entrance to the waterbath have been discussed in an industry guidance document by the Humane Slaughter Association (358) and can be summarised as follows:

- A wet entry ramp that becomes electrified
- Slow line speeds that allow the wing (or other part of the body to enter the water before the head)
- Dipped shackle lines (where the bird descends too gradually and part of the body, e.g., the beak, enters the water first. This can cause the skeletal muscle of the body to contract and the bird loses contact)
- Incorrect angle of the entry ramp
- Agitated birds at the entry to the waterbath
- Physical contact between birds on the shackle line, particularly if the birds are wet, leading to a shock from the adjacent bird

In addition to the pain experienced by the bird, a pre-stun shock may also cause birds to flap, lift their head and miss the stun bath. When deep waterbaths are used, a steeply inclined ramp ascending over the entrance to the water may reduce the number of birds experiencing pre-stun shocks. The ramp must briefly hold birds back at the top of the ramp so they smoothly, but quickly, swing with their head into the water in one motion. It is also important that the ramp is electrically isolated from the bath and water does not flow down its surface as this can also result in birds receiving a shock from the wet material.

PO2.1.3: Electrical parameters required for effective waterbath stunning

A stun is said to be effective if it renders the bird rapidly unconscious and insensible for a period of at least 45 seconds (23), to allow death to occur following neck cutting. Effective electrical stunning involves the application of an electrical current to the brain of sufficient magnitude to induce generalised epilepsy and thus unconsciousness. The aim of any stunning system is to achieve a 100% effective stun. When waterbath stunning is used, the most effective electrical parameters can achieve an effective stun of up to 96% using EEG measurements, and 100% using non-EEG methods. Of all the electrical parameters tested scientifically, neither AC nor DC currents give a 100% stunning effectiveness when using EEG methods of measurement (359). Based on current research, it is impossible to specify an effective minimum current that is appropriate for all the electrical frequencies and waveforms used in commercial establishments. The interaction between the different variables is complex, for example, it has been shown that higher frequencies require higher currents to induce an effective stun (359, 360). There is even doubt as to whether some of the higher frequency currents stun at all. Certain minimum currents relevant to different frequency ranges have been stipulated in Council Regulation (EC) No 1099/2009 (46) (Table 04) and OIE Guidelines (15) (Table 05).

Table 04: Minimum required current per bird as stipulated in Council Regulation (EC) No 1099/2009

Frequency (Hz)	Chickens	Turkeys	Ducks/Geese	Quail
<200	100mA	250mA	130mA	45mA
200 - 400	150mA	400mA	Not permitted	Not permitted
400 - 1500	200mA	400mA	Not permitted	Not permitted

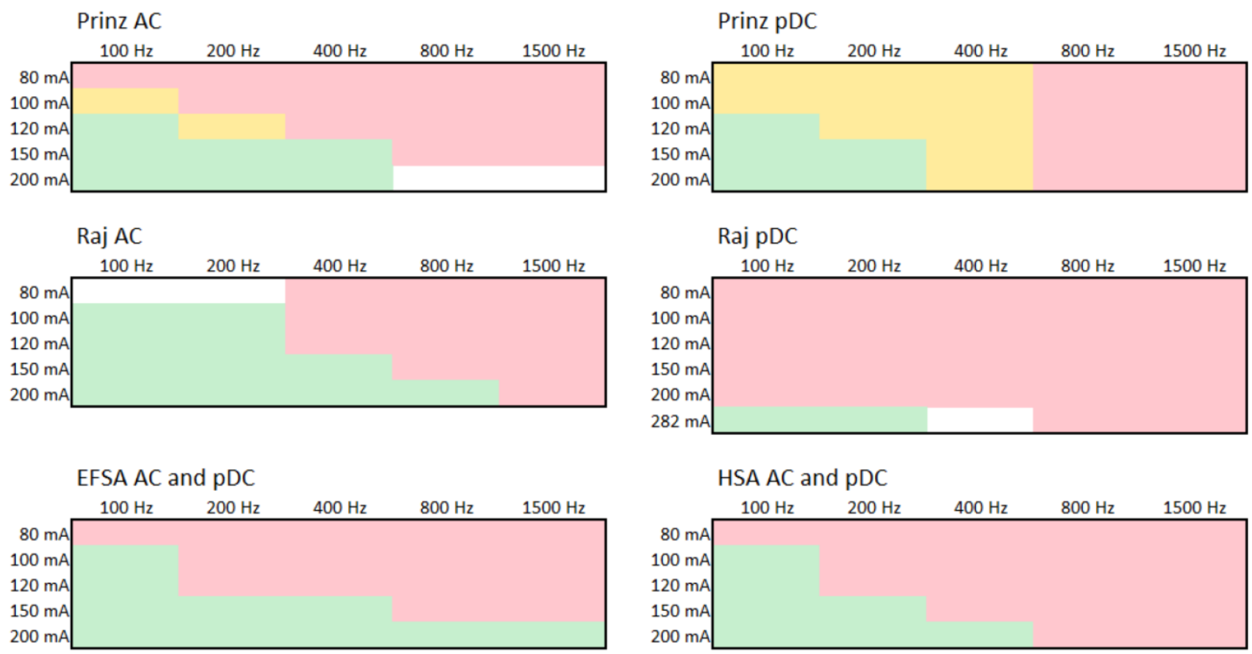
Table 05: Minimum recommended current per bird as stipulated in the OIE Terrestrial Animal Health Code (2019)

Frequency (Hz)	Meat chickens	Laying hens	Turkeys	Ducks/Geese
<50	100mA	100mA	150mA	130mA
50 - 200	100mA	Not specified	250mA	Not specified
200 - 400	150mA	Not specified	400mA	Not specified
400 - 1500	200mA	Not specified	400mA	Not specified

The optimum electrical parameters to produce an effective stun have been described as a low frequency (sinusoidal AC waveform of 50Hz) using a minimum current of 120 mA (chickens) or 150mA (turkeys) (24). These combinations result in the abolition of brain activity and the onset of a quiescent EEG, indicating an unequivocal stun, however they would also result in cardiac arrest in the majority of birds. For carcass quality and market access reasons, electrical systems that produce cardiac arrest are not commonly used in Australia and most systems operate at either high frequency, low voltages (or both) that do not result in the death of the bird. A multi-phase system that is popular in the Australian poultry industry is a two-phase electrical waterbath stunner (Simmons Engineering Company, Dallas, GA, USA) that delivers two consecutive low-voltage stunning phases. In Phase I, a pulsed DC of 550Hz is applied with a low voltage of 12-15V in a shallow brine waterbath. The water depth is approximately 1 cm, with the chickens' head resting on a metal grid. This is immediately followed by Phase II, consisting of a smooth metal plate. In Phase II, a sine wave AC of 50Hz is applied with 20-40V. There is limited research on this system, with that available pre-dating the scope of this review.

Research (pre-2017) on the effectiveness of different electrical parameters used commercially (100Hz to 1500Hz) for stunning meat chickens was summarised in the Farmed Bird Welfare Science Review (2017) (328) and replicated in Figure 03.

Figure 03: Reproduced from the Bird Welfare Science Review (2017) (328)- ***Effective (green), marginal (yellow), ineffective (pink) and untested (white) combinations of frequency and stun current for broilers as determined by Prinz and Raj for AC and pDC currents, and EFSA and HSA recommendations. mA: milliamperes***



For Raj the green squares mark treatments that resulted in at least 80% of the birds displaying signs of unconsciousness. For Prinz the green squares mark treatments where at least 90% of the birds displayed unconsciousness and there was recovery in less than 20% of the birds. The yellow squares mark treatments where only 85% of the birds displayed signs of unconsciousness or there were significant amounts of recovery.

Post-2017 research is limited, although the studies reviewed concur with the findings in Figure 03 A Brazilian study examined the effect of waveform (AC vs. DC) and frequency (300Hz and 650Hz) at 100mA per bird on stunning efficacy and meat quality in broilers (361). Stunning efficacy was assessed using behavioural observations (absence of rhythmic breathing, eye reflex, and coordinated wing flapping) and blood parameters (lactate, glucose, creatine kinase, sodium, and potassium), which were measured after bleeding. When an AC waveform was used, birds stunned at 650 Hz showed higher frequency of eye reflex (14.52%) ($P< 0.05$) compared to those stunned at 300 Hz (3.64%). For the DC waveform, only birds stunned at 650 Hz showed positive eye reflexes (5.45%). Occurrence of eye reflex in birds stunned at 650 Hz in both waveforms showed no significant differences. Other behavioural indicators were only observed in birds stunned using the AC 650 Hz treatment (rhythmic breathing - 3.23% and wing flapping - 1.61%). For blood parameters, there was interaction between frequency and waveform for serum lactate and sodium ($P < 0.05$), with a decrease in concentration seen with the DC 300Hz treatment. The researchers concluded that all treatments were effective, as all the birds displaying a positive eye reflex showed no other signs of recovery and blood parameters were within those considered as basal levels for broilers. The lower frequency DC treatment was deemed to be most effective. Another study on the efficacy of electrical waterbath stunning used physicochemical (pH, peroxides) and histological parameters (glycogen reserve, muscle damages) as stress markers (362). The researchers found that birds stunned with 200mA (800Hz and 100Hz) showed a reduction in the superoxide free radicals (considered a marker of preslaughter stress in the muscle of poultry) when compared with birds that were slaughtered without stunning.

There were no updated research findings for other poultry species. Recommendations for the electrical parameters that should be used for guinea fowl are included in Humane Slaughter Association guidance material (350), which advises the use of low frequency 50Hz system to deliver a minimum electrical current of 100mA per bird. An EFSA report recommended that a current of at least 130 mA per bird (sinusoidal AC at 50 Hz) should be used when stunning ducks in a waterbath stunner (24). The report stipulates that a reversible stun can be achieved by raising the frequency, however, no current/frequency combination above 50Hz which will result in an effective stun has been investigated to-date.

PO2.1.4: Stun duration

A minimum stun duration, during which contact must be maintained, is not stipulated in Australian regulation. The stun duration can be longer depending on the line speed and length of the waterbath. EFSA (360) recommend a minimum stun duration of 4 seconds, however, commercially the application of the stun is much longer than this.

PO2.1.5: Assessment of effective stunning

The most reliable means of determining unconsciousness is by measuring brain activity using EEG signals. Behavioural indicators of insensibility are less reliable (relevant research reviewed in Farmed Bird Welfare Science Review (328)).

Testing of brain-stem reflexes, such as rhythmic breathing and the nictitating membrane reflex can be used as a proxy indicator of unconsciousness, when appropriate electrical parameters are applied (360). At AC currents above 120 mA and at frequencies up to 200Hz, the absence of corneal reflex is closely associated with suppressed EEG and is a reliable indicator of unconsciousness. However, at higher frequencies, direct observations of rhythmic breathing and signs of epilepsy are not sufficiently reliable indicators of unconsciousness (363). This is because the amount of current required to produce unconsciousness is higher than that needed to produce the physical signs in birds; meaning that birds leaving the waterbath appear to be stunned, but may in fact be in a state of electrically induced paralysis. Therefore, in a commercial setting it is very difficult to ascertain whether a bird is effectively stunned or not.

Although it is usual to look for outcomes of unconsciousness in poultry following stunning, the risk of poor welfare can be detected better if monitoring is focused on detecting signs of consciousness. These signs can be monitored at two key stages after electrical waterbath stunning (19).

Stage 1: ***Between the exit from the waterbath stunner and neck cutting***

- Recommended indicators: tonic seizures, breathing and spontaneous blinking.
- Additional indicators: vocalisation, palpebral reflex and corneal reflex.

Stage 2: ***During bleeding***

- Recommended indicators: wing flapping and breathing.
- Additional indicators: spontaneous swallowing, head shaking, palpebral reflex and corneal reflex.

PO2.2: Electrical head-only stunning

In Australia, manual head-only electrical stunning of poultry is relatively uncommon and is normally only used for small numbers of birds on the farm or in low throughput processing establishments. To facilitate stunning, birds are usually restrained in a cone, on a shackle, or held manually by the legs. The electrical stunning current is delivered by a pair of adjustable tongs or fixed electrodes, applied across the head. Much of the research on head-only electrical stunning of poultry pre-dates the scope of this review but is summarised in the Farmed Bird Welfare Science Review (328) and EFSA reports (17, 24). More recently, the concept of a head-only application of electrodes has been evaluated commercially for incorporation into a poultry processing line (364). The manufacturer of this equipment has developed two systems; one where the birds are suspended from the legs in metal shackles before stunning (before being supported by plastic cones during stunning), and a further modification which only shackles birds after stunning has been completed. As this system is a constant current, rather than constant voltage system, it can also potentially eliminate the problem of ineffective stunning of birds seen during conventional water bath stunning and also avoid cardiac arrest (which may be a market requirement) (365). There has also been recent work on the head-only stunning of turkeys (366) as an alternative to electrical waterbath and CAS stunning during seasonal slaughter. It was suggested that further research should investigate the use of a refined electrode shape together with an electrode application in a position that reflects the anatomy of the head (behind the eyes) to increase the likelihood of producing an effective stun. The researchers observed that, unlike waterbath stunning, the subjective assessment of an effective head-only stun reflected the signs of an effective stun in the EEG.

Section summary - Electrical stunning of poultry

There is currently considerable debate among scientists about the reliability of criteria used to determine effective stunning when the current is applied to the whole bird in a water bath. The current could affect peripheral nerves and induce muscle paralysis in a bird that remains conscious. This is not the case with head-only applications.

PO2.3: Controlled atmosphere stunning (CAS)

PO2.3.1: Commercial CAS systems

The use of controlled atmosphere stunning (CAS) has several commercial advantages over electrical waterbath stunning, such as improved carcass quality. However, from an animal welfare perspective, the main advantage of CAS systems is that they do not require conscious birds to be shackled; with birds either conveyed through the system in their transport crates or by means of a moving conveyor (328). In October 2017, McDonald's Corp. made a commitment to source chicken in the U.S. and Canada from facilities using CAS (367). The commercial use of CAS in poultry meat processing facilities is currently limited to chickens and turkeys.

Controlled atmosphere stunning (CAS) methods, involve the exposure of poultry to gas mixtures. Stunning is achieved by the increased concentration of carbon dioxide in air (hypercapnic hypoxia), by the depletion of oxygen (anoxia), or a combination of the two (hypercapnic anoxia), depending on the gas mixtures used. There are three main types of CAS system used commercially:

- Deep pit
- Moving conveyor
- Cabinet

Timings vary with gas concentrations but typically birds may be expected to lose consciousness within 20 s - 1 minute and all the birds may be expected to be dead within 5 minutes (368). The features of each commercial system are summarised in Table 06.

Table 06: Main design features of commercial CAS systems

Type of system	Manufacturer	Presentation of birds	CO2 exposure	Ability to observe birds
Deep pit	Linco Baader	De-stacked transport crates lowered stepwise	Deep pit filled with CO_2. Birds exposed to increasing concentration	Birds screened from view as they descend. Some newer systems fitted with cameras
Moving conveyor	Marel Stork, Anglia Autoflow	Transport crates (Anglia Autoflow, Marel Stork) or birds tipped from de-stacked transport crates onto conveyor (Marel Stork),	Biphasic (two concentrations of CO_2) or multi-stage (different stages of increasing CO_2 concentration)	Windows along the length of the tunnel system to allow birds in each phase to be observed
Cabinet	Meyn	Transport crates in module	CO_2 of increasing concentration is injected into the cabinet	Windows allow birds to be observed throughout the process

For an overall animal evaluation, there are a number of welfare criteria upon which CAS systems can be judged (369):

- Method of handling into the system
- Aversiveness of the gas
- Disruption of respiration
- Period of anaesthesia
- State of consciousness at the onset of wing flapping and muscular contractions
- Effective control of gas concentrations throughout the enclosure
- Effective monitoring of gas concentrations, including the ability to visually observe birds.

The summary of controlled atmosphere stunning provided by the Farmed Bird Welfare Science Review (328) states:

The advantages of controlled atmosphere stunning are that the birds do not need to be handled or positioned and that it is a robust system insensitive to bird size or conformity able to stun and kill 100% of birds. The disadvantages of CAS systems are that during the time it takes to lose consciousness the birds may experience the unpleasant effects of carbon dioxide exposure, and convulsions including wing flapping resulting in broken bones may occur while they have some level of consciousness. There remains uncertainty about how averse chickens are to the respiratory disruption caused by the carbon dioxide and also about the state of consciousness of the birds when they experience convulsions. The risk of these two welfare compromises is minimised by the exposing the birds first to low concentrations of carbon dioxide, possibly with enhanced levels of oxygen for a period of around 1 minute before increasing the carbon dioxide concentration to ensure the birds are killed.

PO2.3.2: Type of gas and application

In all the commercial CAS systems used in Australia, birds are first exposed to relatively low concentrations of carbon dioxide (<40% CO2 by volume in air), and then, once the birds are unconscious, they are exposed to a higher concentration (approximately 80% - 90% CO2 by volume in air) to ensure a stunning effect that lasts until death. Some commercial systems also add oxygen to the CO_2 mixture, usually in an attempt to improve product quality (369). Carbon dioxide can also be used in combination with inert gases, for example, nitrogen or argon. Carbon dioxide is an acidic gas and a potent respiratory stimulant that can cause breathlessness before the loss of consciousness. Poultry have chemoreceptors sensitive to carbon dioxide, and birds will react to exposure, in the form of headshaking and gasping, to the presence of CO_2 at relatively low concentrations. When monitoring the initial stages of CAS (<40%) it is normal to observe open-beak gasping and headshaking prior to birds losing consciousness. During exposure to a concentration of <40%, birds should lose consciousness, as indicated by loss of posture. Wing flapping activity may be observed later in the stunning process: however, this is thought to occur after unconsciousness.

PO2.3.3: Stunning effectiveness

Investigations into the use of CAS systems under practical conditions have shown an excellent stunning effectiveness. An examination of the use of the biphasic system showed that 0.003% of birds were classified as awake after neck cutting, which means one bird out of 36,072 (370). In biphasic CO_2 systems the presence of a heartbeat after stunning has been demonstrated though full recovery of all birds cannot be achieved [Link to ref] (328). There has been little research on the use of CAS in poultry since 2017. One study has investigated the effects of CAS for turkeys (371) using two different recipes of CO2 exposure (Method 1: 30% CO2 15 sec, 55% CO2 40 sec, 70% CO2 45 sec; method 2: 30% CO2 15 sec, 80% CO2 85 sec) on the efficacy of stunning, blood stress indicators and meat quality of turkeys. Stunning was found to be more effective (more unconscious birds) when the CO2 concentration increased rapidly (as in method 2 - biphasic method). In contrast, method 1 (slower rise in concentration of CO2) resulted in a significantly ($P < 0.05$) higher percentage of sensible (respiratory movements and eye reflexes) and semi-sensible. The use of CAS for other poultry species is limited, although there have been investigations into on-farm use which contain some useful information. An EFSA document (372) covering the on-farm slaughter of poultry (for purposes other than slaughter) referred to the use of controlled atmosphere methods for pheasants, indicating that through practical experience a residual oxygen (O_2) of 5% by volume or less created using a mixture of 80% by volume of argon and 20% by volume of carbon dioxide caused death in pheasants within 2 minutes. Controlled atmosphere systems can be used for ducks, where exposure to an inert gas or to <30% Carbon Dioxide and 60% inert gas has resulted in a stunned state (17). However, there is a lack of research on the effects of the induction phase and any aversion before unconsciousness.

Although it is usual to look for outcomes of unconsciousness in poultry following stunning, the risk of poor welfare can be detected better if monitoring is focused on detecting signs of consciousness. These signs can be monitored at two key stages after controlled atmosphere stunning (19).

Stage 1: ***Between the exit from the CAS system and neck cutting (particularly during shackling)***

- Recommended indicators: breathing, muscle tone, wing flapping and spontaneous blinking.
- Additional indicators: corneal or palpebral reflex and vocalisations.

Stage 2: ***During bleeding***

- Recommended indicators: wing flapping, muscle tone and breathing.
- Additional indicators: corneal or palpebral reflex.

PO2.4: Low atmospheric pressure stunning (LAPS)

The summary of LAPS provided by the Farmed Bird Welfare Science Review (328) states:

LAPS has the potential to offer significant welfare benefits for poultry slaughter. Birds remain in their transport crates during LAPS stunning so there is no need for conscious birds to be shackled or positioned. Its effectiveness is relatively insensitive to variations in bird size and conformity so it does not underperform when presented with flocks with a large variance in bird size. No aversive gas is used to displace oxygen, and stunning is irreversible. Concerns surrounding LAPS centre around spasms and wing flapping induced by hypoxia, as well as the potential for hypobaric injury.

Since the completion of the Farmed Bird Welfare Science Review in 2017, the use of LAPS has been approved for use in the EU, after assessment by the ESFA Panel on Animal Health and Welfare (220). The scientific opinion presented by the panel was that the use of LAPS was found to ‘be able to provide a level of animal welfare not lower than that provided by at least one of the currently allowed methods.’ However, this assessment is only valid for meat chickens of <4kg intended for human consumption and under the technical and ambient conditions described in the submission (summarised in Table 07). Deviations from these conditions might have different consequences for animal welfare which were not assessed by the panel. For example, the conclusions from the assessment cannot be extended to other types of chicken (layers, breeders and chicks) and if LAPS methodology is intended to be used for the stunning of layers, further studies would be required to determine the effect of decompression on intra-abdominal shell eggs.

Table 07: Conditions for the use of LAPS as specified by EFSA (220)

Conditions		Requirement
Bird characteristics	Species	Meat chickens
	Live weight	<4kg
Technical conditions	Rate of decompression	The process consists of two phases: in the first phase, the vacuum chamber pressure is reduced from atmospheric pressure to an absolute vacuum pressure of ~ 250 Torr (~ 33 kPa) in ~ 67 s. In the second phase, a sliding gate valve is partially closed, gradually reducing the effective pumping speed by 'choke flow', to a minimum chamber pressure of ~ 150 Torr (~ 20 kPa). The rate of reduction of chamber pressure in the second phase is varied in relation to starting ambient temperature.
	Duration of each phase	See below
	Total exposure time	Total LAPS evacuation process = 280 s, followed by return to atmospheric pressure (recompression cycle is about 20 s)
Ambient conditions	Temperature	Ambient temperature: 11.6 ± 0.3°C (average over the cycles, over 2 days). Additional temperature settings described in a later study (373)
	Humidity	Humidity: 51.8 ± 1.8% Additional humidity conditions described in a later study (373)

Recent research (373) has studied the vacuum characteristics currently used in commercial LAPS, to provide more information on the necessary ambient conditions (Table 07) during commercial operation. The effects of temperature, water, and outgassing (desorption of gases/vapors from the vacuum surfaces or poultry) of water on the process was discussed. The rate of pressure change was determined to be consistent with previous work reporting minimum discomfort and pain for meat chickens during LAPS.
Further research into the effects of LAPS on different sized birds, different species, potential for aversion, and the effect of gas expansion in body cavities was recommended by EFSA, before it could be used as a stunning method more widely. A study on the aversion of LAPS compared with gaseous methods of stunning (CO_2 and Nitrogen) (374). The researchers used trained broiler breeders to indicate aversion to a particular environment, by relinquishing a food reward to seek a preferable environment. They found that cessation of feeding occurred most rapidly in the CO_2 environment, whereas in the low atmospheric pressure and Nitrogen environments, birds continued to eat for longer. Behavioural indicators of possible aversion were also more pronounced in CO_2, with gasping occurring only in the CO_2 exposed animals. Headshaking occurred in all treatments, though with greater frequency and earlier for CO_2 exposed birds. The pathological consequences of LAPS have also recently been investigated (375), to provide information on the possible effects of gas expansion in body cavities during the procedure. A commercial LAPS process was applied and birds were subject to necropsy examination to detect and score (1 to 5, minimal to severe) haemorrhagic lesions or congestion for all major organs and cavities (e.g., air sacs, joints, ears and heart) as well as external assessment for product quality (e.g., wing tips). The results were compared to a control group that had been euthanased by an intravenous injection of pentobarbital sodium. Haemorrhagic lesions were observed in the calvaria, brains, hearts and lungs of both treatment groups, but were more severe in the LAPS treatment group. In the barbiturate group, more severe haemorrhagic lesions were observed in the superficial pectoral muscles as well as greater congestion of the infraorbital sinuses, liver, spleens, duodenum, kidneys and gonads. The findings were used to provide evidence that LAPS did not result in visible changes to the air sacs and intestines

consistent with distension. The researchers also noted that there was no evidence of barotrauma in the ears and sinuses.

Post-2017 research adds to the body of evidence, supporting the use of LAPs during the slaughter of poultry, although additional research on other species and types of poultry is still required. EFSA (220) have also recommended that emergency procedures associated with LAPS system failures (including manufacturer's instructions) should be developed and implemented by Food Business Operators.

Section summary - Use of CAS and LAPS in poultry

An advantage of controlled atmosphere stunning is that the birds do not need to be removed from their transport crates in some systems. CAS is also insensitive to bird size and uniformity, being able to effectively stun almost 100% of birds. With some of the commercial settings, it is an irrecoverable stun, making the stun to stick interval less critical to maintain bird welfare. There is still some uncertainty about the aversiveness of the process and the state of consciousness of the birds when they experience convulsions. Industry and animal welfare certification schemes (33) are managing these risks by requiring birds to be first exposed to low concentrations of carbon dioxide (<40%) until unconsciousness is produced before being introduced to a higher concentration.

The use of LAPS is considered to result in a welfare outcome at least equivalent to the commercial methods of stunning currently used for poultry. Post-2017 research into possible aversion demonstrated that LAPS is likely to be less aversive than the use of CO_2. Pathological data also confirms that organ integrity is not compromised by the LAPS process.

PO3: Slaughter

PO3.1: Neck cutting process

In poultry, the carotid arteries are positioned close to the surface of the neck (376). A complete ventral neck cut at sufficient depth across the front of the neck would sever both carotid arteries. In meat chickens, neck cutting is largely performed using automatic equipment consisting of one or more rapidly rotating blades. It is usually the line speed which determines whether manual or automatic equipment is used, with automatic equipment being favoured on faster processing lines. Poultry species that are commonly slaughtered on slower lines (e.g., turkeys, ducks and geese) are normally slaughtered using a manual neck-cut with a knife. This can either take the form of a ventral neck cut, severing both carotid arteries or a unilateral neck cut, severing one carotid artery and one jugular vein on the side of the neck. The minor poultry species (guinea fowl, pheasant, pigeon, quail or partridge) are sometimes bled using a spear stick, particularly if they are being sold with the head and feathers on. The aim of the spear stick is to sever blood vessels on one side of the neck. If pheasants and partridges are being processed for sale without being exsanguinated then it is imperative that the method used to stun also results in the death of the bird.

PO3.2: Time to brain death

When reversible stunning methods used, the blood vessels severed at neck cutting (and resulting loss of blood) will determine the time to loss of brain activity; with severance of both carotid arteries resulting in a faster decline in EEG activity compared with a unilateral cut severing one carotid artery (24); in chickens (<20s compared to >60s) (cited in (328)). No peer-reviewed literature on the effect of neck cutting methodology on time to brain death was found for guinea fowl, pheasant, pigeon, quail or partridge, however, the general principles for neck cutting in poultry apply.

PO3.3: Slaughter without stunning

In poultry, slaughter without prior stunning may occur to fulfil specific market requirements or during back-up killing when birds have missed the stunner or show signs of recovery on the line. Within Australia, manual neck cutting (without stunning) is the most commonly used back-up method for birds that miss the stunner. The MCoP also permits decapitation as a back-up method (62). The current RSPCA Approved Farming Scheme Standard for Meat Chickens (33) reflects the Model Code and also allows non-stun slaughter as a back-up method. Interestingly, the AUSVET Plan (377) does not recognise the use of decapitation for conscious poultry and only includes it as a permitted terminal procedure for unconscious animals. Researchers have raised concerns about the use of decapitation as a killing method for conscious birds due to the lack of evidence that it produces immediate loss of consciousness and sensibility (cited in (17, 24)

PO4: Animal welfare assessment

PO4.1: Assessment of poultry welfare during lairage and handling

Animal-based measures have been validated for use during the lairage and handling periods, however, some may not be feasible under certain circumstances. For example, birds in the middle of a container may not be visible for assessment, or the fast line speed may make the detection of some measures impossible. A list of animal-based measures with relevant definitions and their related welfare consequence/s are detailed in an EFSA report on poultry slaughter (369). These are summarised for lairage and handling in Table 08.

Table 08: Summary of proposed animal-based measures for the assessment of lairage and handling as detailed in the EFSA report on the slaughter of poultry (369).

Welfare consequence	Suggested animal-based measures
Heat stress	Mortality (DOAs), panting,
Cold stress	Mortality (DOAs), fluffing feathers (piloerection of feathers), huddling, shivering
Fear, pain	Escape behaviour, piling, vocalisations, injuries, muscle contractions (spasms/tremors), wing flapping (prolonged bout)
Restriction of movement	Body positions (insufficient space for all birds to sit),
Prolonged hunger	Bile in crates/on-floor, presence of urates/orange cast in crates/on-floor

PO4.2: Assessment of stunning and slaughter

There is no "gold standard" for assessing effective stunning in poultry, and different researchers use different methods. Parameters to assess the state of insensibility following stunning have included: body posture, rhythmic breathing (monitored through regular cloaca movements), interphalangeal, pedal, palpebral and corneal (nictitating membrane) reflexes (tested through pinching the comb and the touching of the bird's eyelids and cornea with a feather); and cardiac activity. EFSA published a scientific opinion in 2013 (19) which proposed a toolbox of indicators for assessing consciousness in poultry at two key stages of monitoring: (a) between the exit from the waterbath stunner and neck cutting and (b) during bleeding. For gas stunning, the opinion proposed a toolbox of indicators for assessing consciousness in poultry at two key stages of monitoring: (a) during shackling and (b) during bleeding. For slaughter without stunning, a toolbox proposed confirming death prior to entering the scald tank. The animal-based measures detailed in this opinion and in the EFSA report on poultry slaughter (369) are summarised in Table 09.

Table 09: Summary of proposed animal-based measures for the assessment of stunning and slaughter developed from the EFSA report on the slaughter of poultry (369).

Welfare consequence	Suggested animal-based measures
Consciousness or returning to consciousness	Head righting (attempt to raise head), head shaking or wing flapping after stunning, birds retaining or regaining posture after stunning, breathing, spontaneous blinking, swallowing, corneal or palpebral reflex and vocalisations
Respiratory distress	Deep breathing, often with open beak, can be accompanied by stretching the neck (gasping), rapid shaking of the head, most times accompanied by stretching and/or withdrawal movements of the head
Fear and pain	Escape attempts, rapid shaking of the head, most times accompanied by stretching and/or withdrawal movements of the head, injuries, muscle contractions (spasms/tremors), wing flapping (prolonged bout), vocalization, withdrawal reaction (fast avoiding movement of the stimulated part of the body)
Alive at scald	Breathing, the corneal or palpebral reflex, muscle tone, pupil size and bleeding.

Ostriches and emu

OS1: Pre-slaughter handling

OS1.1: Arrival and unloading

Ratites (ostriches and emu) are generally unloaded using a shallow ramp or horizontal unloading bay. They are usually handled individually during the unloading process to manage their speed as they leave the truck and reduce the risk of injury. Using animal-based measures to assess the unloading process for ostriches (See OS5: Animal welfare assessment for a full description), researchers concluded that the unloading process was less stressful than either loading or transport (378), however, during unloading, 10% of birds slipped and 2% of birds fell. In the same study, an increase in the heterophil:lymphocyte ratio [H:L] was attributed to handling, loading and transport stress. The magnitude of the changes in the haematological parameters were not influenced by journey length.

OS1.2: Lairage

In South Africa, ostriches are often transported and lairaged in the cooler part of the day to protect them from high ambient temperatures (379), similar advice is provided for the transport of emu in the Model Code of Practice (380). In ostriches, significant rises in body temperature above the physiological norm for the species can occur during transport, indicating that stress during the journey may affect the bird’s ability to adequately thermoregulate (378). When ratites are held overnight, they are often kept in larger naturally ventilated pens with bedding or sand floors. Lairages often have a unique design, comprising of hexagonal pens to avoid birds being crushed in right-angled corners. The Model Code of Practice for ostriches (381) states that high-sided, solid fencing in holding areas is preferred to reduce the chance of injury and escape.

OS1.3: Handling

Ratite handling methods can differ quite significantly from those seen in other livestock species. Ostriches in particular, are inquisitive birds who prefer to follow the handling operative, rather than being driven from behind (379). Researchers assessing the handling of ostriches, found that the percentage frequencies of falls, slips and aggressions (see OS5: Welfare assessment for detailed definitions) were significantly ($P<0{\cdot}01$) higher during handling than during either loading or unloading (378). The overall proportion of birds that slipped and fell during handling was approximately 60% and 40% respectively. When compared to industry targets for slipping and falling (31) used in other livestock, the researchers deduced that this represented a serious hazard to both bird and handler welfare.

The Model Code of Practice for ostriches (381) refers to the use of hoods and crooks as handling tools. It states that hoods should only be used in birds > 6 months that are being individually handled by a stockperson. There is no research on the use of hoods as it affects animal welfare, however, their use is more commonplace on-farm during husbandry procedures rather than during handling in the abattoir. Crooks have been used to catch ostriches, to restrain the neck and head for hooding (381). Using crooks to catch birds can result in injury and their use is not recommended. Electric goads are not generally used during the handling of ostriches.

OS1.4: Humane killing sick and injured birds

In South Africa, sick and injured ostriches identified at unloading are usually stunned using a head-only electrical stunning system, with manually applied electrical stunning tongs. This is followed by cutting the throat (Leisha Hewitt, personal observation).

OS2: Restraint

OS2.1: Restraint methods

When manually applied electrical stunning tongs were used commercially, ostrich restraint comprised of manually applied leg clamp (or wing supports), and manual holding of the beak (379). With the development of an automated electrical stunning system, restraint became an integral part of the process, applied simultaneously with the stunning mechanism.

OS3: Stunning

OS3.1: Electrical stunning

The processing of ratites is not currently covered in the scope of Australian Standards AS 4696:2007, AS 4465:2006 or AS 4841:2006. However, AS 4841:2006 covering the production of pet meat requires compliance with the relevant model code of practice which for ostriches would be the Model Code of Practice for the Welfare of Animals: Farming of Ostriches, 2003 (381) and for emu is the Model Code of Practice for the Welfare of Animals: Husbandry of Captive Bred Emus, 2001 (380). There is also a specific Australian standard for the processing of ratites, the Australian Standard for Hygienic Production of Ratite (Emu/Ostrich) Meat for Human Consumption, AS 501:2001. This standard covers subjects such as antemortem and postmortem inspection, but does not contain any specific provisions regarding the stunning and slaughter process for ratites. Instead, it stipulates that abattoirs must develop an animal care statement which details how the Model Code of Practice for the Welfare of Animals: slaughtering establishments (62) will be met.

In the past, the electrical stunning of ostriches was commonly undertaken using hand-held electrical stunning tongs, applied either side of the ostrich's head (17, 379, 382). A study on the use of an electrical current of 400mA and 50Hz demonstrated that it effectively stunned ostriches and provided a period of unconsciousness that lasted until death, provided that bleeding was performed within 60 seconds post-stun (379). Subsequently, EFSA stipulated a recommended current for stunning ostriches using a head-only application of 500mA (17), however, it was stated that this was an interim recommendation until further research was carried out. Since that report, an automated system (the Divac Ostrich Stunning Box©) has been developed and is now used throughout the South African ostrich industry. The development of the Divac system was based on the parameters identified in the earlier study (379), being set to deliver a current of 400-800mA for a duration of 10 seconds. The stunning box used to hold the animal during application, rotates through 180 degrees during the stunning process, allowing the ostrich to be shackled and bled in around 20 seconds post stun.

OS3.2: Captive bolt stunning

The use of a captive bolt pistol is recognised in the Model Codes of Practice for ostriches and emu and the Land Transport Standards and Guidelines (42, 380, 381), however, with the exception of shot position (detailed in the Land Transport Standards and Guidelines) there is no further detail around the appropriate parameters for use. Possible factors influencing the effectiveness of captive bolt stunning in ostriches have been cited as the size of the ostrich's head, movement during restraint and fragility of the skull (379), with recommendations against the use of captive bolt pistols as a primary method of stunning. The use of captive bolt pistols is considered to be an appropriate method for killing sick and injured birds, or as a back-up method. The Model Code of Practice for ostriches also recognises firearms (gunshot) as an appropriate method for humane killing of adult birds and neck dislocation or decapitation for chicks. The welfare concerns around the use of neck dislocation and decapitation have not been investigated in ostriches, however, some of the research on the use of these methods in birds is discussed in Section PO1.4 and may be relevant here.

The mechanism of mechanical stunning is not completely understood with ostriches (17). It is not known whether the stunned state is produced by physical destruction of the neural tissue, bleeding of the brain or concussion. Parts of the skull overlying the hemispheres are very thin (especially in Emus), and it is not known whether concussion through impact with the skull could be produced. The recommended shot position for the use of captive bolts in ratites is detailed in the Land Transport Standards and Guidelines, 2012 (42).

OS4: Slaughter

OS4.1: Slaughter methods

The commercial slaughter of ratites involves the application of a manual cut across the ventral surface of the bird's neck close to the head. This type of cut severs all soft tissues ventral to the spine, ensuring that both carotid arteries are cut (379). In commercial abattoirs, the ventral neck cut is often followed by a thoracic stick. There is no evidence in the literature to suggest that the vertebral arteries contribute significantly in terms of blood flow to the brain, hence the necessity of a thoracic stick from a welfare perspective is questionable. In a study of manual head-only electrical stunning of ostriches (using manually applied restraint) (379), the average stun to stick time was 46 s (±6.1). The authors stated that increasing the stun to stick time appeared to increase the chance of recovery.

A major problem with all stunning methods is the post-stun convulsions which can delay the shackling, hoisting and bleeding of the animals and therefore prolong the time to brain death and increase the chance of recovery (17). This can be managed by using a restraint method that physically controls movement of the legs to enable effective shackling.

OS5: Animal welfare assessment

Open beak panting (hyperventilation) and skin temperature are useful indicators of thermal comfort in ratites (379, 383). Use of a corneal reflex test and the absence of rhythmic breathing as indicators of effective stunning and slaughter (379). Researchers have successfully used animal-based measures to assess loading, transport and unloading (378). Measures included falling (if any part of the body other than the toes touched the ground), aggression/fight (antagonistic acts between ostriches or between ostriches and people), kick (when an ostrich made a powerful forward or downward kick), jump (when an ostrich jumped), capture myopathy (when an ostrich fell and refused to stand on its own, even when helped) and slip (when an ostrich lost its balance temporarily, interfering with its normal walking).

Appendices

APEN1: Restraint methods for different species

Red meat animals	
Species	**Typical restraint methods used in Australia**
Cattle	Stun (knocking) box
	Rail/belt
	Electrical stun box
Calves	Stun pen
	V-restrainer
Buffalo	Stun (knocking) box
Pigs	Stun pen
	V-restrainer
	Rail/belt
	Gondola (CAS)
	Stun (knocking) box - Breeders
Sheep	Stun pen
	V-restrainer
Goats	Stun pen
	V-restrainer
Horses	Stun (knocking) box
Donkeys	Stun (knocking) box
Camels	Stun (knocking) box
Alpacas	Stun pen
	Stun (knocking) box
Rabbits	Shackles
	Manual

Poultry	
Species	**Typical restraint methods used in Australia**
Chickens (meat)	Shackles
	Cone
	Transport crate
Chickens (layer)	Shackles
	Cone
	Transport crate
Turkeys	Shackles
	Cone
	Transport crate
Ducks	Shackles
	Cone
Geese	Shackles
	Cone
Quail	Shackles
Ostrich	Stun (knocking) box
	Electrical stun box
Emu	Stun (knocking) box
Guinea fowl	Shackles
	Cone
Pheasant	Shackles
	Cone
Partridge	Manual
Pigeon	Manual

APEN2: Stunning methods for different species

Red meat animals	
Species	**Typical stunning methods used in Australia**
Cattle	Non-penetrative captive bolt
	Penetrative captive bolt
	Electrical
	Free bullet
Calves	Electrical
	Penetrative captive bolt
Buffalo	Penetrative captive bolt
	Non-penetrative captive bolt
	Electrical
Pigs	Controlled Atmosphere (CO_2)
	Electrical
	Penetrative captive bolt
Sheep	Electrical
	Penetrative captive bolt
Goats	Electrical
	Penetrative captive bolt
Horses	Penetrative captive bolt
	Free bullet
Donkeys	Penetrative captive bolt
	Free bullet
Camels	Penetrative captive bolt
Alpacas	Penetrative captive bolt
Rabbits	Electrical
	Neck dislocation

Poultry	
Species	**Typical stunning methods used in Australia**
Chickens (meat)	Electrical waterbath
	Controlled Atmosphere (CO_2)
	Neck dislocation
	Electrical head-only
Chickens (layer)	Electrical waterbath
	Controlled Atmosphere (CO_2)
	Neck dislocation
	Electrical head-only
Turkeys	Electrical waterbath
	Controlled Atmosphere (CO_2)
	Neck dislocation
	Electrical head-only
Ducks	Electrical waterbath
	Neck dislocation
	Electrical head-only
Geese	Electrical waterbath
	Neck dislocation
	Electrical head-only
Quail	Electrical waterbath
	Decapitation
Ostrich	Penetrative captive bolt
	Electrical
Emu	Penetrative captive bolt
	Electrical
Guinea fowl	Neck dislocation
	Decapitation
Pheasant	Neck dislocation
	Decapitation
Partridge	Neck dislocation
	Decapitation
Pigeon	Neck dislocation
	Decapitation

APEN3: Slaughter methods for different species

Red meat animals	
Species	**Typical slaughter methods used in Australia**
Cattle	Neck stick
	Thoracic stick
Calves	Neck stick
	Thoracic stick
Buffalo	Neck stick
	Thoracic stick
Pigs	Thoracic stick
Sheep	Neck stick
Goats	Neck stick
Horses	Neck stick
Donkeys	Neck stick
Camels	Neck stick
Alpacas	Neck stick
Rabbits	Neck stick

Poultry	
Species	**Typical slaughter methods used in Australia**
Chickens (meat)	Neck cut - Ventral
	Neck cut - bilateral
Chickens (layer)	Neck cut - Ventral
	Neck cut - bilateral
Turkeys	Neck cut - Ventral
	Neck cut - bilateral
Ducks	Neck cut - Ventral
	Neck cut - bilateral
Geese	Neck cut - Ventral
	Neck cut - bilateral
Quail	Neck cut - unilateral
	Decapitation
Ostrich	Neck cut - Ventral
Emu	Neck cut - Ventral
Guinea fowl	Neck cut - unilateral
	Decapitation
Pheasant	Neck cut - unilateral
	Decapitation
Partridge	Neck cut - unilateral
	Decapitation
Pigeon	Neck cut - unilateral
	Decapitation

References

1. Oudyn T, Davis, J., Balazs, C., Molloy, C., & Roberts, M. . It's hoped an upcoming change to the law that regulates the slaughter of cattle for meat will see Victoria to become the first state to allow mobile abattoirs. ABC News Victoria (ABC1 Melbourne). 2018.
2. Animal welfare in abattoirs, poultry processors and knackeries - regulatory scorecard. 2021. RSPCA Australia PO Box 265 Deakin West ACT 2600.
3. Farm Animal Welfare Council (FAWC). Report on the Welfare of Farmed Animals at Slaughter or Killing Part 1: Red Meat Animals. 2003.
4. Coleman GJ, Hemsworth PH. Training to improve stockperson beliefs and behaviour towards livestock enhances welfare and productivity. Revue Scientifique Et Technique-Office International Des Epizooties. 2014;33(1):131-7.
5. Coleman GJ, Rice M, Hemsworth PH. Human-animal relationships at sheep and cattle abattoirs. Animal Welfare. 2012;21:15-21.
6. Hemsworth PH, Rice M, Karlen MG, Calleja L, Barnett JL, Nash J, et al. Human-animal interactions at abattoirs: Relationships between handling and animal stress in sheep and cattle. Applied Animal Behaviour Science. 2011;135(1-2):24-33.
7. Von Holleben K, Von Wenzlawowicz M, Gregory N, Anil H, Velarde A, Rodriguez P, et al. Report on good and adverse practices - Animal welfare concerns in relation to slaughter practices from the viewpoint of veterinary sciences2010 2/8/2012; 1.3:[81 p.]. Available from: http://www.dialrel.eu/images/veterinary-concerns.pdf.
8. Broom DM. The Evolution of pain. Vlaams DiergeneeskTijdschr. 2001;70:17-21.
9. Grandin T. Livestock-handling assessments to improve the welfare of cattle, pigs and sheep. Animal Production Science. 2018;58(3):403-7.
10. Livestock handling and transport / edited by Temple Grandin, Department of Animal Sciences, Colorado State University, USA2014 2014. 484- pages p.
11. Grandin T. Making Slaughterhouses More Humane for Cattle, Pigs, and Sheep. Annual Review of Animal Biosciences, Vol 1. 12013. p. 491-512.
12. Grandin T. Improving animal welfare: a practical approach. Grandin T, editor2009.
13. Cruz Ldl, Gibson TJ, Guerrero-Legarreta I, Napolitano F, Mora-Medina P, Mota-Rojas D. The welfare of water buffaloes during the slaughter process: a review. Livestock Science. 2018;212:22-33.
14. Bourguet C, Deiss V, Tannugi CC, Terlouw EMC. Behavioural and physiological reactions of cattle in a commercial abattoir: Relationships with organisational aspects of the abattoir and animal characteristics. Meat Science. 2011;88(1):158-68.
15. World Organisation for Animal Health (OIE). 2021. – Terrestrial Animal Health Code, OIE, Paris
16. Zeman A. Consciousness. Brain. 2001. 124:1263-89.
17. European Food Safety Authority. The welfare aspects of the main systems of stunning and killing applied to commercially farmed deer, goats, rabbits, ostriches, ducks, geese and quail. EFSA Journal. 2006;326:1-18.
18. Verhoeven MTW, Gerritzen MA, Hellebrekers LJ, Kemp B. Indicators used in livestock to assess unconsciousness after stunning: a review. Animal. 2015;9(2):320-30.
19. European Food Safety Authority. Scientific Opinion on monitoring procedures at slaughterhouses for poultry. EFSA Journal. 2013;11(12).
20. European Food Safety Authority. Scientific Opinion on monitoring procedures at slaughterhouses for bovines. EFSA Journal. 2013;11(12).
21. European Food Safety Authority. Scientific Opinion on monitoring procedures at slaughterhouses for pigs. EFSA Journal. 2013;11(12).

22. European Food Safety Authority. Scientific Opinion on monitoring procedures at slaughterhouses for sheep and goats. EFSA Journal. 2013;11(12).

23. EFSA proposes indicators for monitoring welfare at slaughter. Vet Rec. 2013;173(4):83.

24. European Food Safety Authority. Welfare aspects of the main systems of stunning and killing the main commercial species of animals. EFSA Journal. 2004;45:1-29.

25. Dalmau A, Temple D, Rodriguez P, Llonch P, Velarde A. Application of the Welfare Quality® protocol at pig slaughterhouses. 2009:497-505.

26. Grandin T. Auditing animal welfare at slaughter plants. Meat Science. 2010;86(1):56-65.

27. Rocha LM, Velarde A, Dalmau A, Saucier L, Faucitano L. Can the monitoring of animal welfare parameters predict pork meat quality variation through the supply chain (from farm to slaughter)? Journal of Animal Science. 2016;94(1):359-76.

28. Manteuffel G, Puppe B, Schon PC. Vocalization of farm animals as a measure of welfare. Applied Animal Behaviour Science. 2004;88(1-2):163-82.

29. Martinez-Miro S, Tecles F, Ramon M, Escribano D, Hernandez F, Madrid J, et al. Causes, consequences and biomarkers of stress in swine: an update. Bmc Veterinary Research. 2016;12.

30. Mellor DJ, Cook CJ, Stafford KJ. Quantifying some responses to pain as a stressor. in: Moberg G P et al [ed] The Biology of Animal Stress: Basic Principles and Implications for Welfare CAB International, Wallingford, Oxon, UK, 171- 198. 2000.

31. AMIC. National Animal Welfare Standards for Livestock Processing Establishments. Crows Nest NSW: Australian Meat Industry Council; 2009.

32. RSPCA. RSPCA Approved Farming Scheme Standard - Pigs. November 2018.

33. RSPCA Approved Farming Scheme Standard - Meat Chickens. 2020.

34. 'It's now time to make CCTV in abattoirs compulsory'. Veterinary Record. 2017;180(24):585-.

35. Nielsen BL, Dybkjaer L, Herskin MS. Road transport of farm animals: effects of journey duration on animal welfare. Animal. 2011;5(3):415-27.

36. Knowles TG, Brown SN, Warriss PD, Phillips AJ, Dolan SK, Hunt P, et al. Effects on sheep of transport by road for up to 24 hours. Vet Rec. 1995;136(17):431-8.

37. Probst JK, Spengler Neff A, Leiber F, Kreuzer M, Hillmann E. Gentle touching in early life reduces avoidance distance and slaughter stress in beef cattle. Applied Animal Behaviour Science. 2012;139(1–2):42-9.

38. NAMI Recommended handling guidelines and audit guide: A systematic approach to animal welfare. September 2019 Rev.2.

39. Sutherland M, McDonald A, McGlone JJ. Effects of variations in the environment, length of journey, and type of trailer on the mortality and morbidity of pigs transported to slaughter. Veterinary Record. 2009;256:13-8.

40. Ritter MJ, Ellis M, Brinkman J, DeDecker JM, Keffaber KK, Kocher ME, et al. Effect of floor space during transport of market-weight pigs on the incidence of transport loss-es at the packing plant and the relationships between transport conditions and losses. Animal Science. 2006;84:2856-64.

41. Australian Standard for the Hygienic Production and Transport of Meat and Meat Products for Human Consumption. AS4696-2007

42. Australian Animal Welfare Standards and Guidelines - Land Transport. 2012.

43. More S, Bicout D, Botner A, Butterworth A, Calistri P, Depner K, et al. Animal welfare aspects in respect of the slaughter or killing of pregnant livestock animals (cattle, pigs, sheep, goats, horses). Efsa Journal. 2017;15(5).

44. Dalmau, A. Velarde, A. Lairage and handling. In: A. Velarde, M. Raj, editors. Animal Welfare at Slaughter. Sheffield, UK: 5 m Publishing; 2016. p. 51–70.

45. Faucitano L. Preslaughter handling practices and their effects on animal welfare and pork quality. Journal of Animal Science. 2018;96(2):728-38.

46. Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing, (2009).

47. Weeks CA. A review of welfare in cattle, sheep and pig lairages, with emphasis on stocking rates, ventilation and noise. Animal Welfare. 2008;17(3):275-84.

48. Iulietto MF, Sechi P, Gaudenzi CM, Grispoldi L, Ceccarelli M, Barbera S, et al. Noise assessment in slaughterhouses by means of a smartphone app. Italian Journal of Food Safety. 2018;7(2):79-82.

49. Grandin T. Improving Welfare and Reducing Stress on Animals at Slaughter Plants. 2014. 421-50 p.

50. Grandin website: https://www.grandin.com/references/bison.paper.html.

51. Grandin T. Handling and Welfare of Livestock in Slaughter Plants. Grandin T, editor. 2007. 329-53 p.

52. Biss ME, Hathaway SC. Effect of pre-slaughter washing of lambs on the microbiological and visible contamination of the carcases. Veterinary Record. 1996;138(4):82-6.

53. Byrne CM, Bolton DJ, Sheridan JJ, McDowell DA, Blair IS. The effects of preslaughter washing on the reduction of *Escherichia coli* O157:H7 transfer from cattle hides to carcasses during slaughter. Letters in Applied Microbiology. 2000;30:142-5.

54. Gill CO. Visible contamination on animals and carcasses and the microbiological conditon of meat. Journal of Food Protection. 2004;67(2):413-9.

55. Bray AR, Graafhuis AE, Chrystall BB. The cumulative effect of nutritional, shearing and preslaughter washing stresses on the quality of lamb meat. Meat Science. 1989;25(1):59-67.

56. Petersen GV. The effect of swimming lambs and subsequent resting periods on the ultimate ph of meat. Meat Science. 1983;9(4):237-46.

57. Walker P, Warner R, Weston P, Kerr M, editors. Effect of washing cattle pre-slaughter on glycogen levels of M. semitendinosus and M. semimembranosus International Congress of Meat Science and Technology; 1999; Japan.

58. Preston F, Wilkes M, McGilchrist P, Pitchford W, editors. Pre-slaughter hide washing affects meat quality in pasture finished cattle, 64th International Congress of Meat Science and Technology Melbourne August 12-17, 2018; 2018.

59. Preston F, Wilkes M, McGilchrist P, Pitchford W, editors. How’s it hanging? Head down behavior in beef cattle increases in response to pre-slaughter hide washing and is associated with increased dark cutting incidence. International Congress of Meat Science and Technology; 2019; Berlin.

60. Grandin T. Auditing animal welfare and making practical improvements in beef-, pork- and sheep-slaughter plants. Animal Welfare. 2012;21:29-34.

61. Grandin T. Auditing and assessing the welfare of livestock and poultry during pre-slaughter handling and stunning2020. 175-92 p.

62. Model Code of Practice for the Welfare of Animals - Livestock at Slaughtering Establishments. SCARM Report 79

63. Shaw NA. The neurophysiology of concussion. Progress in Neurobiology. 2002;67(4):281-344.

64. Terlouw C, Bourguet C, Deiss V. Consciousness, unconsciousness and death in the context of slaughter. Part I. Neurobiological mechanisms underlying stunning and killing. Meat Sci. 2016;118:133-46.

65. Borzuta K, Lisiak D, Janiszewski P, Grzeskowiak E. The physiological aspects, technique and monitoring of slaughter procedures and their effects on meat quality - a review. Annals of Animal Science. 2019;19(4):857-73.

66. Blumenfeld H, Taylor J. Why do Seizures Cause Loss of Consciousness? The Neuroscientist. 2003;9(5):301-10.

67. Blumenfeld H. Cellular and Network Mechanisms of Spike-Wave Seizures. Epilepsia. 2005;46(s9):21-33.

68. Humane Slaughter Association. Electrical stunning of red meat animals. 2016. The Old School, Brewhouse Hill, Wheathampstead, Herts, AL4 8AN, UK.

69. Warriss PD. Exsanguination of animals at slaughter and the residual blood content of meat. Veterinary Record 1984. 1984;115(12)::292-5 ; 37 ref.

70. Gregory NG. Bowhunting deer. Animal Welfare. 2005; 14:111-6.

71. Levinger IM. Schechita in the light of the year 2000. Jerusalem, Maskil David. 1995.

72. Fuseini A, Wotton SB, Hadley PJ, Knowles TG. The compatibility of modern slaughter techniques with halal slaughter: a review of the aspects of 'modern' slaughter methods that divide scholarly opinion within the Muslim community. Animal Welfare. 2017;26(3):301-10.

73. Fuseini A, Knowles TG, Hadley PJ, Wotton SB. Halal stunning and slaughter: Criteria for the assessment of dead animals. Meat Science. 2016;119:132-7.

74. Rosen SD. Physiological insights into Shechita. Veterinary Record. 2004;154(24):759

75. Gibson TJ, Johnson CB, Murrell JC, Chambers JP, Stafford KJ, Mellor DJ. Components of electroencephalographic responses to slaughter in halothane-anaesthetised calves: Effects of cutting neck tissues compared with major blood vessels. New Zealand Veterinary Journal. 2009;57(2):84-9.

76. Gibson TJ, Johnson CB, Murrell JC, Hulls CM, Mitchinson SL, Stafford KJ, et al. Electroencephalographic responses of halothane-anaesthetised calves to slaughter by ventral-neck incision without prior stunning. New Zealand Veterinary Journal. 2009;57(2):77-83.

77. Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Amelioration of electroencephalographic responses to slaughter by non-penetrative captive-bolt stunning after ventral-neck incision in halothane-anaesthetised calves. New Zealand Veterinary Journal. 2009;57(2):96-101.

78. Zulkifli I, Goh YM, Norbaiyah B, Sazili AQ, Lotfi M, Soleimani AF, et al. Changes in blood parameters and electroencephalogram of cattle as affected by different stunning and slaughter methods in cattle. Animal Production Science. 2014;54(2):187-93.

79. Sabow AB, Goh YM, Zulkifli I, Ab Kadir MZ, Kaka U, Adeyemi KD, et al. Electroencephalographic and blood parameters changes in anaesthetised goats subjected to slaughter without stunning and slaughter following different electrical stunning methods. Animal Production Science. 2019;59(5):849-60.

80. Barrasso R, Bonerba E, Ceci E, Roma R, Alo A, Mottola A, et al. Evaluation of the animal welfare during religious slaughtering. Italian Journal of Food Safety. 2020;9(1):39-43.

81. Bozzo G, Barrasso R, Marchetti P, Roma R, Samoilis G, Tantillo G, et al. Analysis of Stress Indicators for Evaluation of Animal Welfare and Meat Quality in Traditional and Jewish Slaughtering. Animals. 2018;8(4).

82. Ceci E, Marchetti P, Samoilis G, Sportelli S, Roma R, Barrasso R, et al. Determination of plasmatic cortisol for evaluation of animal welfare during slaughter. Italian Journal of Food Safety. 2017;6(3):134-7.

83. Imlan JC, Kaka U, Goh Y-M, Idrus Z, Awad EA, Abubakar AA, et al. Effects of Slaughter Knife Sharpness on Blood Biochemical and Electroencephalogram Changes in Cattle. Animals. 2020;10(4).

84. Gregory NG, Fielding HR, von Wenzlawowicz M, von Holleben K. Time to collapse following slaughter without stunning in cattle. Meat Science. 2010;85(1):66-9.

85. Gibson TJ, Dadios N, Gregory NG. Effect of neck cut position on time to collapse in halal slaughtered cattle without stunning. Meat Science. 2015;110:310-4.

86. Bozzo G, Bonerba E, Barrasso R, Roma R, Luposella F, Zizzo N, et al. Evaluation of the Occurrence of False Aneurysms During Halal Slaughtering and Consequences on the Animal's State of Consciousness. Animals. 2020;10(7).

87. Gregory NG, Schuster P, Mirabito L, Kolesar R, McManus T. Arrested blood flow during false aneurysm formation in the carotid arteries of cattle slaughtered with and without stunning. Meat Science. 2012;90(2):368-72.

88. Gregory NG, Shaw FD, Whitford JC, Patterson-Kane JC. Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. Meat Science. 2006;74(4):655-7.

89. Gregory NG, von Wenzlawowicz M, Alam RM, Anil HM, sildere TY, Silva-Fletcher A. False aneurysms in carotid arteries of cattle and water buffalo during shechita and halal slaughter. Meat Science. 2008;79(2):285-8

90. Neves JEG, Paranhos da Costa MJR, Roca RO, Faucitano L, Gregory NG. A note comparing the welfare of Zebu cattle following three stunning-slaughter methods. Meat Science. 2016;117:41-3.

91. Agbeniga B, Webb EC. Effect of slaughter technique on bleed-out, blood in the trachea and blood splash in the lungs of cattle. South African Journal of Animal Science. 2012;42(5):524-9.

92. Anil MH, Yesildere T, Aksu H, Matur E, McKinstry JL, Weaver HR, et al. Comparison of Halal slaughter with captive bolt stunning and neck cutting in cattle: exsanguination and quality parameters. Animal Welfare. 2006;15(4):325-30.

93. Anil M, Yesildere T, Aksu H, Matur E, McKinstry J, Erdogan O, et al. Comparison of religious slaughter of sheep with methods that include pre-slaughter stunning, and the lack of differences in exsanguination, packed cell volume and meat quality parameters. Animal Welfare. 2004;13(4):387-92.

94. Khalid R, Knowles TG, Wotton SB. A comparison of blood loss during the Halal slaughter of lambs following Traditional Religious Slaughter without stunning, Electric Head-Only Stunning and Post-Cut Electric Head-Only Stunning. Meat Science. 2015;110:15-23.

95. Bakhsh A, Ismail I, Hwang Y-H, Lee J-G, Joo S-T. Comparison of Blood Loss and Meat Quality Characteristics in Korean Black Goat Subjected to Head-Only Electrical Stunning or without Stunning. Korean Journal for Food Science of Animal Resources. 2018;38(6):1286-93.

96. Gregory NG, von Wenzlawowicz M, von Holleben K. Blood in the respiratory tract during slaughter with and without stunning in cattle. Meat Science. 2009;82(1):13-6.

97. Lambooij E, van der Werf JTN, Reimert HGM, Hindle VA. Restraining and neck cutting or stunning and neck cutting of veal calves. Meat Science. 2012;91(1):22-8.

98. Costa LN, Lo Fieg DR, Tassone F. Relationship between pre-slaughter handling and carcass bruising in calves. Italian Journal of Animal Science. 2005;4:257-9.

99. Kline HC, Edwards-Callaway LN, Grandin T. Short Communication: Field observation: Pen stocking capacities for overnight lairage of finished steers and heifers at a commercial slaughter facility. Applied Animal Science. 2019;35(1):130-3.

100. Klinglmair K, Stevens KB, Gregory NG. Luminance and glare in indoor cattle-handling facilities. Animal Welfare. 2011;20(2):263-9.

101. Willson DW, Baier FS, Grandin T. An observational field study on the effects of changes in shadow contrasts and noise on cattle movement in a small abattoir. Meat Science. 2021;179.

102. Grigor PN, Cockram MS, Steele WB, McIntyre J, Williams CL, Leushuis IE, et al. A comparison of the welfare and meat quality of veal calves slaughtered on the farm with those subjected to transportation and lairage. Livestock Production Science. 2004;91(3):219-28.

103. Hikmah H, Abustam, Asja MA, Nahariah N, Iop. The blood plasma profile of Bali Cattle with different resting statuses before slaughter. 1st International Conference of Animal Science and Technology. IOP Conference Series-Earth and Environmental Science. 2472019.

104. Chulayo AY, Bradley G, Muchenje V. Effects of transport distance, lairage time and stunning efficiency on cortisol, glucose, HSPA1A and how they relate with meat quality in cattle. Meat Science. 2016;117:89-96.

105. Costa FdO, Brito G, Manuel Soares de Lima J, Sant'Anna AC, Rodrigues Paranhos da Costa MJ, del Campo M. Lairage time effect on meat quality in Hereford steers in rangeland conditions. Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science. 2019;48.

106. del Campo Gigena M, Soares de Lima JM, Brito G, Manteca X, Hernandez P, Montossi F. Effect of Finishing Diet and Lairage Time on Steers Welfare in Uruguay. Animals. 2021;11(5).

107. del Campo M, Brito G, de Lima JS, Hernandez P, Montossi F. Finishing diet, temperament and lairage time effects on carcass and meat quality traits in steers. Meat Science. 2010;86(4):908-14.

108. Romero MH, Uribe-Velasquez LF, Sanchez JA, Miranda-de la Lama GC. Risk factors influencing bruising and high muscle pH in Colombian cattle carcasses due to transport and pre-slaughter operations. Meat Science. 2013;95(2):256-63.

109. Liotta L, Costa LN, Chiofalo B, Ravarotto L, Chiofalo V. Effect of lairage duration on some blood constituents and beef quality in bulls after long journey. Italian Journal of Animal Science. 2007;6(4):375-84.

110. Brown-Brandl TM, Eigenberg RA, Nienaber JA. Water spray cooling during handling of feedlot cattle. International Journal of Biometeorology. 2010;54(6):609-16.

111. Chen JM, Schutz KE, Tucker CB. Sprinkler flow rate affects dairy cattle avoidance of spray to the head, but not overall, in an aversion race. Applied Animal Behaviour Science. 2016;179:23-31.

112. Mpamhanga CJ, Wotton SB. The effects of pre-slaughter restraint (for the purpose of cattle identification) on post-slaughter responses and carcass quality following the electrical stun/killing of cattle in a Jarvis Beef stunner. Meat Science. 2015;107(0):104-8.

113. Pighin DG, Davies P, Pazos AA, Ceconi I, Cunzolo SA, Mendez D, et al. Biochemical profiles and physicochemical parameters of beef from cattle raised under contrasting feeding systems and pre-slaughter management. Animal Production Science. 2015;55(10):1310-7.

114. Reiche AM, Oberson JL, Silacci P, Messadene-Chelali J, Hess HD, Dohme-Meier F, et al. Pre-slaughter stress and horn status influence physiology and meat quality of young bulls. Meat Science. 2019;158.

115. Hultgren J, Algers B, Arvidsson Segerkvist K, Berg C, Karlsson AH. Animal handling and stress-related behaviour at mobile slaughter of cattle. Preventive veterinary medicine. 2020;177.

116. Croney CC, Wilson LL, Curtis SE, Cash EH. Effects of handling aids on calf behavior. Applied Animal Behaviour Science. 2000;69(1):1-13.

117. Grandin T. Cattle vocalizations are associated with handling and equipment problems at beef slaughter plants. Applied Animal Behaviour Science. 2001;71(3):191-201.

118. Ceballos MC, Sant'Anna AC, Boivin X, Costa FdO, Carvalhal MVdL, Paranhos da Costa MJR. Impact of of good practices of handling training on beef cattle welfare and stockpeople attitudes and behaviors. Livestock Science. 2018;216:24-31.

119. Stilwel G, de Carvalho C, Lima MS, Broom DM. The effect of duration of manual restraint during blood sampling on plasma cortisol levels in calves. Animal Welfare. 2008;17(4):383-5.

120. Andrade O, Orihuela A, Solano J, Galina CS. Some effects of repeated handling and the use of a mask on stress responses in Zebu cattle during restraint. Applied Animal Behaviour Science. 2001;71(3):175-81.

121. Mitchell KD, Stookey JM, Laturnas DK, Watts JM, Haley DB, Huyde T. The effects of blindfolding on behavior and heart rate in beef cattle during restraint. Applied Animal Behaviour Science. 2004;85(3-4):233-45.

122. Gilliam JN, Shearer JK, Bahr RJ, Crochik S, Woods J, Hill J, et al. Evaluation of brainstem disruption following penetrating captive-bolt shot in isolated cattle heads: comparison of traditional and alternative shot-placement landmarks. Animal Welfare. 2016;25(3):347-53.

123. Vecerek V, Kamenik J, Voslarova E, Volfova M, Machovcova Z, Konvalinova J, et al. The Impact of Deviation of the Stun Shot from the Ideal Point on Motor Paralysis in Cattle. Animals. 2020;10(2).

124. Vecerek V, Voslarova E, Kamenik J, Machovcova Z, Valkova L, Volfova M, et al. The effect of slaughtering skills on the welfare of cattle during stunning with a captive bolt. Acta Veterinaria Brno. 2021;90(1):109-16.

125. Collins SL, Kull J, Benham C, Krawczel P, Donohue KD, Caldwell M. Comparison of penetrating and non-penetrating captive bolt in an alternative occipital approach in calves. Animal Welfare. 2020;29(1):59-67.

126. Gouveia KG, Ferreira PG, da Costa JCR, Vaz-Pires P, da Costa PM. Assessment of the efficiency of captive-bolt stunning in cattle and feasibility of associated behavioural signs. Animal Welfare. 2009;18(2):171-5.

127. Bartz B, Collins M, Stoddard G, Appleton A, Livingood R, Sobcynski H, et al. Assessment of nonpenetrating captive bolt stunning followed by electrical induction of cardiac arrest in veal calves. Journal of Animal Science. 2015;93(9):4557-63.

128. Gibson TJ, Johnson CB, Murrell JC, Mitchinson SL, Stafford KJ, Mellor DJ. Electroencephalographic responses to concussive non-penetrative captive-bolt stunning in halothane-anaesthetised calves. New Zealand Veterinary Journal. 2009;57(2):90-5.

129. Octavio Oliveira SE, Dalla Costa FA, Gibson TJ, Dalla Costa OA, Coldebella A, Gregory NG. Evaluation of brain damage resulting from penetrating and non-penetrating stunning in Nelore Cattle using pneumatically powered captive bolt guns. Meat Science. 2018;145:347-51.

130. Octavio Oliveira SE, Gregory NG, Dalla Costa FA, Gibson TJ, Rodrigues Paranhos da Costa MJ. Efficiency of low versus high airline pressure in stunning cattle with a pneumatically powered penetrating captive bolt gun. Meat Science. 2017;130:64-8.

131. Octavio Oliveira SE, Gregory NG, Dalla Costa FA, Gibson TJ, Dalla Costa OA, Rodrigues Paranhos da Costa MJ. Effectiveness of pneumatically powered penetrating and non-penetrating captive bolts in stunning cattle. Meat Science. 2018;140:9-13.

132. Casagrande RR, Alexander L, Edwards-Callaway LN. Effects of penetrating captive bolt gun model and number of stuns on stunning-related variables of cattle in a commercial slaughter facility. Meat Science. 2020;170.

133. Gibson TJ, Octavio Oliveira SE, Dalla Costa FA, Gregory NG. Electroencephalographic assessment of pneumatically powered penetrating and non-penetrating captive-bolt stunning of bulls. Meat Science. 2019;151:54-9.

134. Kline HC, Wagner DR, Edwards-Callaway LN, Alexander LR, Grandin T. Effect of captive bolt gun length on brain trauma and post-stunning hind limb activity in finished cattle Bos taurus. Meat Science. 2019;155:69-73.

135. Wagner DR, Kline HC, Martin MS, Alexander LR, Grandin T, Edwards-Callaway LN. The effects of bolt length on penetration hole characteristics, brain damage and specified-risk material dispersal in finished cattle stunned with a penetrating captive bolt stunner. Meat Science. 2019;155:109-14.

136. Martin MS, Kline HC, Wagner DR, Alexander LR, Edwards-Callaway LN, Grandin T. Evaluation of different captive bolt lengths and breed influence upon post-stun hind limb and forelimb activity in fed cattle at a commercial slaughter facility. Meat Science. 2018;143:159-64.

137. Wotton SB, Gregory NG, Whittington PE, Parkman ID. Electrical stunning of cattle. Veterinary Record. 2000;147(24):681-4.

138. Weaver AL, Wotton SB. The Jarvis Beef Stunner: Effects of a prototype chest electrode. Meat Science. 2009;81(1):51-6.

139. McLean D, Meers L, Ralph J, Owen JS, Small A. Development of a microwave energy delivery system for reversible stunning of cattle. Research in Veterinary Science. 2017;112:13-7.

140. Rault JL, Hemsworth PH, Cakebread PL, Mellor DJ, Johnson CB. Evaluation of microwave energy as a humane stunning technique based on electroencephalography (EEG) of anaesthetised cattle. Animal Welfare. 2014;23(4):391-400.

141. Small A, Lea J, Niemeyer D, Hughes J, McLean D, McLean J, et al. Development of a microwave stunning system for cattle 2: Preliminary observations on behavioural responses and EEG. Research in Veterinary Science. 2019;122:72-80.

142. Kim G-D, Lee H-S, Jung E-Y, Lim H-J, Seo H-W, Lee Y-H, et al. The effects of CO2 gas stunning on meat quality of cattle compared with captive bolt stunning. Livestock Science. 2013;157(1):312-6.

143. Limon G, Guitian J, Gregory NG. An evaluation of the humaneness of puntilla in cattle. Meat Science. 2010;84(3):352-5.

144. Limon G, Guitian J, Gregory NG. A review of the humaneness of puntilla as a slaughter method. Animal Welfare. 2012;21(S2):3-8.

145. Horcada A, Juarez M, Valera M, Bartolome E. Using infrared ocular thermography as a tool to predict meat quality from lean cattle breeds prior to slaughter: Exploratory trial. Spanish Journal of Agricultural Research. 2019;17(4).

146. Terlouw EMC, Bourguet C, Deiss V, Mallet C. Origins of movements following stunning and during bleeding in cattle. Meat Science. 2015;110:135-44.

147. Vecerek V, Kamenik J, Voslarova E, Vecerkova L, Machovcova Z, Volfova M, et al. The occurrence of reflexes and reactions in cattle following stunning with a captive bolt at the slaughterhouse. Animal Science Journal. 2020;91(1).

148. Napolitano F, De Rosa G, Grasso F, Pacelli C, Bordi A. Influence of space allowance on the welfare of weaned buffalo (Bubalus bubalis) calves. Livestock Production Science. 2004;86(1-3):117-24.

149. Hoogesteijn R, Hoogesteijn A. Conflicts between cattle ranching and large predators in Venezuela: could use of water buffalo facilitate felid conservation? Oryx. 2008;42(1):132-8.

150. Chandra BS, Das N. The handling and short-haul road transportation of spent buffaloes in relation to bruising and animal welfare. Tropical Animal Health and Production. 2001;33(2):155-63.

151. McCorkell R, Wynne-Edwards K, Galbraith J, Schaefer A, Caulkett N, Boysen S, et al. Transport versus on-farm slaughter of bison: Physiological stress, animal welfare, and avoidable trim losses. Canadian Veterinary Journal-Revue Veterinaire Canadienne. 2013;54(8):769-74.

152. Meichtry C, Glauser U, Glardon M, Ross SG, Lechner I, Kneubuehl BP, et al. Assessment of a specifically developed bullet casing gun for the stunning of water buffaloes. Meat Science. 2018;135:74-8.

153. Gregory NG, Spence JY, Mason CW, Tinarwo A, Heasman L. Effectiveness of poll stunning water buffalo with captive bolt guns. Meat Science. 2009;81(1):178-82.

154. Small A, White R. Developing Appropriate Stunning Methods for Halal Slaughter of Water Buffalo. Wagga Wagga, NSW Australia, AgriFutures Australia; 2020.

155. Ahsan M, Hasan B, Algotsson M, Sarenbo S. Handling and Welfare of Bovine Livestock at Local Abattoirs in Bangladesh. Journal of Applied Animal Welfare Science. 2014;17(4):340-53.

156. Alam MR, Islam MJ, Amin A, Shaikat AH, Pasha MR, Doyle RE. Animal-Based Welfare Assessment of Cattle and Water Buffalo in Bangladeshi Slaughterhouses. Journal of Applied Animal Welfare Science. 2020;23(2):219-30.

157. KS S-G, Faucitano L, Dadgar S, Shand P, LA G, Crowe TG. Road transport of cattle, swine and poultry in North America and its impact on animal welfare, carcass and meat quality: a review. Meat Science. 2012;92:227-43.

158. Urrea VM, Bridi AM, Ceballos MC, da Costa M, Faucitano L. Behavior, blood stress indicators, skin lesions, and meat quality in pigs transported to slaughter at different loading densities. Journal of Animal Science. 2021;99(6).

159. Velarde A, Raj M, Grandin T. Animal Welfare at Slaughter: 5m Publishing, Sheffield.; 2016.

160. Dalmau A, Nande A, Velarde A, Guido Di M, Juliana CRR, Halinen-Elemo K, et al. Application of the Welfare Quality® protocol in pig slaughterhouses of five countries. Livestock science. 2016;193:78-87.

161. Dalla Costa OA, Dalla Costa FA, V F, Dos Santos Lopes L. Risk factors associated with pig preslaughtering losses. Meat Science. 2019;155:61-8.

162. L F. Preslaughter handling practices and their effects on animal welfare and pork quality. Journal of Animal Science. 2018;96:728-38.

163. Arduini A, Redaelli V, Luzi F, Dall'Olio S, Pace V, Costa LN. Relationship between Deck Level, Body Surface Temperature and Carcass Damages in Italian Heavy Pigs after Short Journeys at Different Unloading Environmental Conditions. Animals. 2017;7(2).

164. Haley C, Dewey CE, T W, R F. Association between in-transit losses, internal trailer temperature, and distance travelled by Ontario market hogs. Canadian Journal of Veterinary Research. 2008;72:385–9.

165. Pereira TL, Titto EAL, Conte S, Devillers N, Sommavilla R, Diesel T, et al. Application of a ventilation fan-misting bank on pigs kept in a stationary trailer before unloading: Effects on trailer microclimate, and pig behaviour and physiological response. Livestock Science. 2018;216:67-74.

166. Fox J. Water sprinkling market pigs in a stationary trailer pre- and post-transport: effects on pig behaviour, gastrointestinal tract temperature and trailer micro-climate2014. 118-24 p.

167. Fox J, Mainau E, Nannoni E, Gonyou HW, Brown JA, Faucitano L, et al. Water sprinkling market pigs in a stationary trailer. 1. Effects on pig behaviour, gastrointestinal tract temperature and trailer micro-climate. Livestock science. 2014;160:113-23.

168. Nannoni E, Weschenfelder AV, Martelli G, Gonyou H, Fox J, Faucitano L, et al. Water sprinkling market pigs in a stationary trailer. 2. Effects on selected exsanguination blood parameters and carcass and meat quality variation. Livestock science. 2014;160:124-31.

169. Velarde A, Dalmau A. Animal welfare assessment at slaughter in Europe: Moving from inputs to outputs. Meat Science. 2012;92(3):244-51.

170. Garcia A, McGlone JJ. Loading and Unloading Finishing Pigs: Effects of Bedding Types, Ramp Angle, and Bedding Moisture. Animals. 2015;5(1).

171. L F, S G. Transport to slaughter and associated handling. In: (ed.) MS, editor. Advances in Pig Welfare. London: Woodhead Publishing; 2018. p. 261–93.

172. Lewis CRG, Hulbert LE, McGlone JJ. Novelty causes elevated heart rate and immune changes in pigs exposed to handling, alleys, and ramps. Livestock Science. 2008;116(1):338-41.

173. Novak BL, Young JM, Newman DJ, Johnson AK, Wagner SA. A ramp in nursery housing affects nursery pig behavior and speeds loading of market hogs. Applied Animal Science. 2020;36(4):574-81.

174. L F, C P. Reception and unloading of animals. In: Velarde A and Raj M (eds.). Animal Welfare at Slaughter. Sheffield, UK: 5 m Publishing; 2016. p. 33–50.

175. Peterson E, Remmenga M, AD H, Akkina JE. Use of temperature, humidity, and slaughter condemnation data to predict increases in transport losses in three classes of swine and resulting foregone revenue. Frontiers Veterinary Science. 2017;4:1-12.

176. Pilcher CM, Mike Rojo-Gomez A, Curtis SE, Wolter BF, Peterson CM, Peterson BA, et al. Effects of floor space during transport and journey time on indicators of stress and transport losses of marketweight pigs. Journal of Animal Science. 2011;89:3809–18.

177. ED E, HAM S, I H, H H. Lying characteristics as determinants for space requirements in pigs. Applied Animal Behaviour Science. 2003;80:19-30.

178. Velarde A, Dalmau A. 10 - Slaughter of pigs. In: Špinka M, editor. Advances in Pig Welfare: Woodhead Publishing; 2018. p. 295-322.

179. Warriss PD. Optimal lairage times and conditions for slaughter pigs: a review. Veterinary Record. 2003;153(6):170-6.

180. Rabaste C, Faucitano L, Saucier L, Mormede P, Correa JA, Giguere A, et al. The effects of handling and group size on welfare of pigs in lairage and their influence on stomach weight, carcass microbial contamination and meat quality. Canadian Journal of Animal Science. 2007;87(1):3-12.

181. Grandin T. Effect of animal welfare audits of slaughter plants by a major fast food company on cattle handling and stunning practices. Journal of the American Veterinary Medical Association. 2000;216(6):848-51.

182. Peeters E, Geers R. Influence of provision of toys during transport and lairage on stress responses and meat quality of pigs. Animal Science. 2006;82:591-5.

183. Faucitano L. Invited Review: effects of lairage and slaughter conditions on animal welfare and pork quality. Canadian Journal of Animal Science.90:461-9.

184. Vermeulen L, Van de Perre V, Permentier L, De Bie S, Verbeke G, Geers R. Sound levels above 85 dB pre-slaughter influence pork quality. Meat Science. 2015;100:269-74.

185. Zhen SB, Liu YR, Li XM, Ge KS, Chen H, Li C, et al. Effects of lairage time on welfare indicators, energy metabolism and meat quality of pigs in Beijing. Meat Science. 2013;93(2):287-91.

186. Pérez M, Palacio J, Santolaria P, Aceña C, Chacon G, Verde M, et al. Influence of lairage time on some welfare and meat quality parameters in pigs. Veterinary research. 2002;33:239-50.

187. Lopez-Arjona M, Escribano D, Mateo SV, Contreras-Aguilar MD, Rubio CP, Tecles F, et al. Changes in oxytocin concentrations in saliva of pigs after a transport and during lairage at slaughterhouse. Research in Veterinary Science. 2020;133:26-30.

188. Lewis CRG, McGlone JJ. Moving finishing pigs in different group sizes: Cardiovascular responses, time, and ease of handling. Livestock Science. 2007;107(1):86-90.

189. Sommavilla R, Titto EAL, Titto CG, Hotzel MJ. Ninety one-days-old piglets recognize and remember a previous aversive handler. Livestock Science. 2016;194:7-9.

190. Jongman EC, Woodhouse R, Rice M, Rault JL. Pre-slaughter factors linked to variation in responses to carbon dioxide gas stunning in pig abattoirs. Animal. 2021;15(2).

191. Hemsworth PH, Barnett JL, Hofmeyr C, Coleman GJ, Dowling S, Boyce J. The effects of fear of humans and pre-slaughter handling on the meat quality of pigs. Australian Journal of Agricultural Research. 2002;53(4):493-501.

192. Correa JA, Torrey S, Devillers N, Laforest JP, Gonyou HW, Faucitano L. Effects of different moving devices at loading on stress response and meat quality in pigs. Journal of Animal Science. 2010;88(12):4086-93.

193. E. BM, W. GH, J. ID, F. RL, J. JD, R. WJ, et al. Effect of animal handling method on the incidence of stress response in market swine in a model system. journal of Animal Science. 2001;79:279.

194. McGlone J, McPherson R, Anderson D. Moving devices for finishing pigs: efficacy of electric prod, board, paddle, or flag. The Professional Animal Scientist. 2004;20:518–23.

195. Coleman GJ, McGregor M, Hemsworth PH, Boyce J, Dowling S. The relationship between beliefs, attitudes and observed behaviours of abattoir personnel in the pig industry. Applied Animal Behaviour Science. 2003;82(3):189-200.

196. Schaeperkoetter MA, Grandin T, Edwards-Callaway L. PSIII-16 Evaluating the impact of group stunning on physiological parameters of pigs in a small abattoir. Journal of Animal Science. 2019;97(Suppl 3):185.

197. Atkinson S, Velarde A, Llonch P, Algers B. Assessing pig welfare at stunning in Swedish commercial abattoirs using CO2 group-stun methods. Animal Welfare. 2012;21(4):487-95.

198. Sindhøj E, Lindahl C, Bark L. Review: Potential alternatives to high-concentration carbon dioxide stunning of pigs at slaughter. Animal. 2021;15(3).

199. Steiner AR, Flammer SA, Beausoleil NJ, Berg C, Bettschart-Wolfensberger R, Pinillos RG, et al. Humanely ending the life of animals: research priorities to identify alternatives to carbon dioxide. Animals. 2019;9(11):911-.

200. Mota-Rojas D, Bolanos-Lopez D, Concepcion-Mendez M, Ramirez-Telles J, Roldan-Santiago P, Flores-Peinado S, et al. Stunning Swine with CO2 Gas: Controversies Related to Animal Welfare. International Journal of Pharmacology. 2012;8(3):141-51.

201. Nowak B, Mueffling Tv, Hartung J. Can CO2 stunning meet welfare of slaughter pigs? Aland A, editor2007. 1038-43 p.

202. Nowak B, Mueffling TV, Hartung J. Effect of different carbon dioxide concentrations and exposure times in stunning of slaughter pigs: Impact on animal welfare and meat quality. Meat science. 2007;75(2):290-8.

203. Verhoeven M, Gerritzen M, Velarde A, Hellebrekers L, Kemp B. Time to loss of consciousness and its relation to Behavior in slaughter Pigs during stunning with 80 or 95% carbon Dioxide. Frontiers in Veterinary Science. 2016;3.

204. Anil MH, Whittington PE, McKinstry JL. The effect of the sticking method on the welfare of slaughter pigs. Meat Science. 2000;55(3):315-9.

205. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, et al. Welfare of pigs at slaughter. EFSA Journal. 2020;18(6).

206. Raj ABM, Gregory NG. Welfare Implications of the Gas Stunning of Pigs 2. Stress of Induction of Anaesthesia. Animal Welfare. 1996;5(1):71-8.

207. Martoft L., Lombolt L., Kolthof C., Rodriguez B.E., Juzen E.W., Jorgensen P.F., et al. Effect of CO2 anesthesia an central nervous system activity in swine. Laboratory Animals. 2002;36:115-26.

208. McKinstry JL, Anil MH. The effect of repeat application of electrical stunning on the welfare of pigs. Meat Science. 2004;67(1):121-8.

209. Brandt P, Margit Dall A. Welfare measurements of finishing pigs on the day of slaughter: A review. Meat science. 2015;103:13-23.

210. Gavinelli A, Kennedy T, Simonin D. The application of humane slaughterhouse practices to large-scale culling. Revue Scientifique Et Technique-Office International Des Epizooties. 2014;33(1):291-301.

211. Galvin JW, Blokhuis H, Chimbombi MC, Jong D, Wotton S. Killing of animals for disease control purposes. Rev Sci Tech Off Int Epizoot. 2005;24(2):711-22.

212. Velarde A, Gispert M, Faucitano L, Manteca X, Diestre A. Survey of the effectiveness of stunning procedures used in Spanish pig abattoirs. Veterinary Record. 2000;146(3):65-8.

213. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, et al. Welfare of pigs at slaughter. Efsa Journal. 2020;18(6).

214. Lambooij E and Algers B IVA, Raj M (eds.) Mechanical stunning and killing methods. Animal Welfare at Slaughter: 5M Publishing, Sheffield, UK; 2016. p. 91–110.

215. Association HS. Captive bolt stunning of livestock. Copyright HSA 2016 The Old School, Brewhouse Hill, Wheathampstead, Herts, AL4 8AN, UK Tel:+44(0)1582 831919 Fax: +44(0)1582 831414 Email: info@hsaorguk Website: wwwhsaorguk2016.

216. Anderson K, Ries E, Backes J, Bishop K, Boll M, Brantner E, et al. Relationship of captive bolt stunning location with basic tissue measurements and exposed cross-sectional brain area in cadaver heads from market pigs1. Translational Animal Science. 2019;3(4):1405-9.

217. Gibson TJ, Mason CW, Spence JY, Barker H, Gregory NG. Factors Affecting Penetrating Captive Bolt Gun Performance. Journal of Applied Animal Welfare Science. 2015;18(3):222-38.

218. Grist A, Bock R, Knowles TG, Wotton SB. Further Examination of the Performance of Blank Cartridges Used in Captive Bolt Devices for the Pre-Slaughter Stunning of Animals. Animals. 2020;10(11).

219. Grist A, Lines JA, Bock R, Knowles TG, Wotton SB. An Examination of the Performance of Blank Cartridges Used in Captive Bolt Devices for the Pre-Slaughter Stunning and Euthanasia of Animals. Animals. 2019;9(8).

220. Health EPanel oA, Welfare, More S, Bicout D, Bøtner A, Butterworth A, et al. Low atmospheric pressure system for stunning broiler chickens. EFSA Journal. 2017;15(12):e05056.

221. Bouwsema JA, Lines JA. Could low atmospheric pressure stunning (LAPS) be suitable for pig slaughter? A review of available information. Animal Welfare. 2019;28(4):421-32.

222. Anil HM, Whittington PE, McKinstry JL. The effect of the sticking method on the welfare of slaughter pigs. Meat Science. 2000;55:315-9.

223. Wotton SB, H. AM, E. WP, L. MJ. Pig slaughtering procedures: head-to-back stunning. Meat Science. 1992;32:245-55.

224. Wotton SB, Gregory NG. Pig slaughtering procedures: time to loss of brain responsiveness after exsanguination or cardiac arrest. Research in Veterinary Science 1986;40:148-51.

225. Grandin T. Developing measures to audit welfare of cattle and pigs at slaughter. Animal Welfare. 2012;21(3):351-6.

226. Di Martino G, Zamprogna S, Garbo A, Caucci C, Marchesan M, Bonfanti L, et al. Welfare assessment in Italian pig slaughterhouses. Large Animal Review. 2017;23(4):149-53.

227. Dalmau A, Geverink NA, Van Nuffel A, van Steenbergen L, Van Reenen K, Hautekiet V, et al. Repeatability of lameness, fear and slipping scores to assess animal welfare upon arrival in pig slaughterhouses. Animal. 2010;4(5):804-9.

228. Reimert I, Bolhuis JE, Kemp B, Rodenburg TB. Indicators of positive and negative emotions and emotional contagion in pigs. Physiol Behav. 2013;109:42-50.

229. Schon PC, Puppe B, Manteuffel G. Automated recording of stress vocalisations as a tool to document impaired welfare in pigs. Animal Welfare. 2004;13(2):105-10.

230. McLennan KM, Miller AL, Dalla Costa E, Stucke D, Corke MJ, Broom DM, et al. Conceptual and methodological issues relating to pain assessment in mammals: The development and utilisation of pain facial expression scales. Applied Animal Behaviour Science. 2019;217:1-15.

231. Mota-Rojas D, Orihuela A, Martinez-Burnes J, Gomez J, Mora-Medina P, Alavez B, et al. Neurological modulation of facial expressions in pigs and implications for production. Journal of Animal Behaviour and Biometeorology. 2020;8(4):232-43.

232. Parotat S, von Holleben K, Arnold S, Troeger K, Luecker E. Hot-water spraying is a sensitive test for signs of life before dressing and scalding in pig abattoirs with carbon dioxide (CO2) stunning. Animal. 2016;10(2):326-32.

233. Broom DM, Goode JA, Hall SJG, Lloyd DM, Parrot RF. Hormonal and physiological effects of a 15 hour road journey in sheep: Comparison with the responses to loading, handling and penning in the absence of transport. British Veterinary Journal. 1996;152:593-604.

234. Kim D, Song JI, Jeon J. Effects of Handling and Transport Vehicle on Stress and Carcass Quality of Market Pigs. The Korean Society for Livestock Housing anf Environment. 2011;17(2):101-6.

235. Jongman EC, Edge MK, Butler KL, Cronin GM. Reduced space allowance for adult sheep in lairage for 24 hours limits lying behaviour but not drinking behaviour. Australian Journal of Experimental Agriculture. 2008;48(6-7):1048-51.

236. Liu HW, Zhong RZ, Zhou DW, Sun HX, Zhao CS. Effects of lairage time after road transport on some blood indicators of welfare and meat quality traits in sheep. Journal of Animal Physiology and Animal Nutrition. 2012;96(6):1127-35.

237. Yalcintan H, Akin PD, Ozturk N, Avanus K, Muratoglu K, Kocak O, et al. Effect of lairage time after 2 h transport on stress parameters and meat quality characteristics in Kivircik ewe lambs. Small Ruminant Research. 2018;166:41-6.

238. Liste G, Miranda-de la Lama GC, Campo MM, Villarroel M, Muela E, Maria GA. Effect of lairage on lamb welfare and meat quality. Animal Production Science. 2011;51(10):952-8.

239. Li X, Xia A-q, Chen L-j, Du M-t, Chen L, Kang N, et al. Effects of lairage after transport on post mortem muscle glycolysis, protein phosphorylation and lamb meat quality. Journal of Integrative Agriculture. 2018;17(10):2336-44.

240. Najafi MH, Mohammadi Y, Najafi A, Shamsolahi M, Mohammadi H. Lairage time effect on carcass traits, meat quality parameters and sensory properties of Mehraban fat-tailed lambs subjected to short distance transportation. Small Ruminant Research. 2020;188.

241. Ekiz B, Ekiz EE, Kocak O, Yalcintan H, Yilmaz A. Effect of pre-slaughter management regarding transportation and time in lairage on certain stress parameters, carcass and meat quality characteristics in Kivircik lambs. Meat Science. 2012;90(4):967-76.

242. Jacob RH, Pethick DW, Chapman HM. Muscle glycogen concentrations in commercial consignments of Australian lamb measured on farm and post-slaughter after three different lairage periods. Australian Journal of Experimental Agriculture. 2005;45(5):543-52.

243. Toohey ES, Hopkins DL. Effects of lairage time and electrical stimulation on sheep meat quality. Australian Journal of Experimental Agriculture. 2006;46(6-7):863-7.

244. Karaca S, Erdogan S, Kor D, Kor A. Effects of pre-slaughter diet/management system and fasting period on physiological indicators and meat quality traits of lambs. Meat Science. 2016;116:67-77.

245. Leme TMdC, Titto EAL, Titto CG, Amadeu CCB, Fantinato Neto P, Vilela RA, et al. Influence of transportation methods and pre-slaughter rest periods on cortisol level in lambs. Small Ruminant Research. 2012;107(1):8-11.

246. Teke B, Ekiz B, Akdag F, Ugurlu M, Ciftci G. Effect of lairage time after short distance transport on some biochemical stress parameters and meat quality of Karayaka lambs. Large Animal Review. 2018;24(1):41-4.

247. Chulayo AY, Muchenje V. The Effects of Pre-slaughter Stress and Season on the Activity of Plasma Creatine Kinase and Mutton Quality from Different Sheep Breeds Slaughtered at a Smallholder Abattoir. Asian-Australas J Anim Sci. 2013;26(12):1762-72.

248. Chulayo AY, Muchenje V. Effect of pre-slaughter conditions on physico-chemical characteristics of mutton from three sheep breeds slaughtered at a smallholder rural abattoir. South African Journal of Animal Science. 2013;43(5):S64-S8.

249. East IJ, Foreman I. The structure, dynamics and movement patterns of the Australian sheep industry. Australian Veterinary Journal. 2011;89(12):477-89.

250. Hemsworth PH, Rice M, Borg S, Edwards LE, Ponnampalam EN, Coleman GJ. Relationships between handling, behaviour and stress in lambs at abattoirs. Animal. 2019;13(6):1287-96.

251. Starling MJ, Payne E, McGreevy P. Optimal Flow-A Pilot Study Balancing Sheep Movement and Welfare in Abattoirs. Animals. 2021;11(2).

252. Apple JK, Kegley EB, Galloway DL, Wistuba TJ, Rakes LK. Duration of restraint and isolation stress as a model to study the dark-cutting condition in cattle. Journal of Animal Science. 2005;83(5):1202-14.

253. Anil MH. Effects of slaughter method on carcass and meat characteristics in the meat of cattle and sheep. Kenilworth, UK: AHDB Beef & Lamb. 2012.

254. Njisane YZ, Muchenje V. Quantifying avoidance-related behaviour and bleeding times of sheep of different ages, sex and breeds slaughtered at a municipal and a commercial abattoirs. South African Journal of Animal Science. 2013;43(5):S38-S42.

255. Bates LSW, Ford EA, Brown SN, Richards GJ, Hadley PJ, Wotton SB, et al. A comparison of handling methods relevant to the religious slaughter of sheep. Animal Welfare. 2014;23:251-8.

256. Finnie JW, Blumbergs PC, Manavis J, Summersides GE, Davies RA. Evaluation of brain damage resulting from penetrating and non-penetrating captive bolt stunning using lambs. Australian Veterinary Journal. 2000;78(11):775-8.

257. Finnie JW, Manavis J, Blumbergs PC, Summersides GE. Brain damage in sheep from penetrating captive bolt stunning. Australian Veterinary Journal. 2002;80(1-2):67-9.

258. Gregory NGe. Animal Welfare and Meat Production. Wallingford, Oxon, UK CAB International; 2007.

259. Berg C, Nordensten C, Hultgren J, Algers B. The effect of stun duration and level of applied current on stun and meat quality of electrically stunned lambs under commercial conditions. Animal Welfare. 2012;21:131-8.

260. Llonch P, Rodríguez P, Casal N, Carreras R, Muñoz I, Dalmau A, et al. Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats. Research in Veterinary Science. 2015;98(0):154-61.

261. Velarde A, Ruiz-de-la-Torre JL, Stub C, Diestre A, Manteca X. Factors affecting the effectiveness of head-only electrical stunning in sheep. Veterinary Record. 2000;147(2):40-3.

262. Linares MB, Bornez R, Vergara H. Cortisol and catecholamine levels in lambs: Effects of slaughter weight and type of stunning. Livestock Science. 2008;115(1):53-61.

263. Rodriguez P, Dalmau A, Manteca X, Litvan H, Jensen EW, Velarde A. Assessment of aversion and unconsciousness during exposure to carbon dioxide at high concentration in lambs. Animal Welfare. 2016;25:73-82.

264. Bornez R, Linares MB, Vergara H. Effects of stunning with different carbon dioxide concentrations and exposure times on suckling lamb meat quality. Meat Science. 2009;81(3):493-8.

265. Bornez R, Linares MB, Vergara H. Systems stunning with CO2 gas on Manchego light lambs: Physiologic responses and stunning effectiveness. Meat Science. 2009;82(1):133-8.

266. Vergara H, Bornez R, Linares MB. CO2 stunning procedure on Manchego light lambs: Effect on meat quality. Meat Science. 2009;83(3):517-22.

267. Small A, McLean D, Keates H, Owen JS, Ralph JP. Preliminary investigations into the use of microwave energy for reversible stunning of sheep. Animal Welfare. 2013;22:291-6.

268. Njisane YZ, Muchenje V. Influence of Municipal Abattoir Conditions and Animal-related Factors on Avoidance-related Behaviour, Bleeding Times at Slaughter and the Quality of Lamb Meat. Asian-Australasian Journal of Animal Sciences. 2013;26(10):1496-503.

269. Cannas S, Palestrini C, Canali E, Cozzi B, Ferri N, Heinzl E, et al. Thermography as a Non-Invasive Measure of Stress and Fear of Humans in Sheep. Animals. 2018;8(9).

270. Kannan G, Terrill TH, Kouakou B, Gelaye S, Amoah EA. Simulated preslaughter holding and isolation effects on stress responses and live weight shrinkage in meat goats. Journal of Animal Science. 2002;80(7):1771-80.

271. Alves e Dias CP, Vieira e Silva F, Matos AM, dos Santos Oliveira LL, Freitas de Oliveira AM, Rocha Junior VR, et al. Performance and carcass characteristics of goats fed crude glycerin in the feedlot and during pre-slaughter lairage. Revista Brasileira De Zootecnia-Brazilian Journal of Animal Science. 2018;47.

272. Matos AM, Silva FV, Oliveira LLS, Borges I, Ruas JRM, Novaes CG, et al. Strategies for the supply of crude glycerin for kids in pre-slaughter lairage. Small Ruminant Research. 2018;167:87-91.

273. Collins SL, Caldwell M, Hecht S, Whitlock BK. Comparison of penetrating and nonpenetrating captive bolt methods in horned goats. American Journal of Veterinary Research. 2017;78(2):151-7.

274. Hullinger P, Stull C. The emergency euthanasia of sheep and goats. UC Davis Veterinary Medicine Extension, School of Veterinary Medicine California1999.

275. Troeger KF. Schlachten: Tierschutz und Fleischqualität. Gegenwartige Praxis – Welche Forderungen sind zu stellen? . leischwirtschaft International. 1990;70:266-72.

276. Levinger IM. Untersuchungen zum Schächtproblem. 1961.

277. Dai F, Dalla Costa A, Bonfanti L, Caucci C, Di Martino G, Lucarelli R, et al. Positive Reinforcement-Based Training for Self-Loading of Meat Horses Reduces Loading Time and Stress-Related Behavior. Frontiers in Veterinary Science. 2019;6.

278. Nivelle B, Vermeulen L, Van Beirendonck S, Van Thielen J, Driessen B. Horse Transport to Three South American Horse Slaughterhouses: A Descriptive Study. Animals. 2020;10(4).

279. Australia S. AS 5340:2020 Livestock loading/unloading ramps and forcing pens.

280. Messori S, Visser EK, Buonanno M, Ferrari P, Barnard S, Borciani M, et al. A tool for the evaluation of slaughter horse welfare during unloading. Animal Welfare. 2016;25(1):101-13.

281. Corrales-Hernandez A, Mota-Rojas D, Guerrero-Legarreta I, Roldan-Santiago P, Rodriguez-Salinas S, Yanez-Pizana A, et al. Physiological responses in horses, donkeys and mules sold at livestock markets. International Journal of Veterinary Science and Medicine. 2018;6(1):97-102.

282. Micera E, Moramarco AM, Zarrilli A. Reduction of the olfactory cognitive ability in horses during preslaughter: Stress-related hormones evaluation. Meat Science. 2012;90:272-5.

283. Gibson TJ, Bedford EM, Chancellor NM, Limon G. Pathophysiology of free-bullet slaughter of horses and ponies. Meat Sci. 2015;108:120-4.

284. Cáraves, M. Gallo, C. Characterization and evaluation of the stunning systems used for horses in Chile. Arch. Med. Vet. 2007;39, 2.

285. Svete AN, Cebulj-Kadunc N, Frangez R, Kruljc P. Serum cortisol and haematological, biochemical and antioxidant enzyme variables in horse blood sampled in a slaughterhouse lairage, immediately before stunning and during exsanguination. Animal. 2012;6(8):1300-6.

286. Werner M, Gallo C. Effects of transport, lairage and stunning on the concentrations of some blood constituents in horses destined for slaughter. Livestock Science. 2008;115(1):94-8.

287. Miranda-de la Lama GC, Gonzales-Castro CA, Gutierrez-Pina FJ, Villarroel M, Maria GA, Estevez-Moreno LX. Welfare of horses from Mexico and the United States of America transported for slaughter in Mexico: Fitness profiles for transport and pre-slaughter logistics. Preventive Veterinary Medicine. 2020;180.

288. Miranda-de la Lama GC, Gonzalez-Castro CA, Gutierrez-Pina FJ, Villarroel M, Maria GA, Estevez-Moreno LX. Horse welfare at slaughter: A novel approach to analyse bruised carcasses based on severity, damage patterns and their association with pre-slaughter risk factors. Meat Science. 2021;172.

289. Roy RC, Riley CB, Stryhn H, Dohoo I, Cockram MS. Infrared Thermography for the Ante Mortem Detection of Bruising in Horses Following Transport to a Slaughter Plant. Frontiers in Veterinary Science. 2019;5.

290. Padalino B, Raidal SL. Effects of Transport Conditions on Behavioural and Physiological Responses of Horses. Animals. 2020;10(1).

291. Mojto J, Slamecka J, Hell P, Zaujec K. Influence of killing methods on the quality of fallow deer meat. Fleischwirtschaft. 2007;87(10):118-20.

292. Pollard JC, Littlejohn RP, Asher GW, Pearse AJT, Stevenson-Barry JM, McGregor SK, et al. A comparison of biochemical and meat quality variables in red deer (Cervus elaphus) following either slaughter at pasture or killing at a deer slaughter plant. Meat Science. 2002;60(1):85-94.

293. Abeyesinghe SM, Goddard PJ. The preferences and behaviour of farmed red deer (Cervus elaphus) in the presence of other farmed species. Applied Animal Behaviour Science. 1998;56(1):59-69.

294. Abeyesinghe SM, Goddard PJ, Cockram MS. The behavioural and physiological responses of farmed red deer (Cervus elaphus) penned adjacent to other species. Applied Animal Behaviour Science. 1997;55(1-2):163-75.

295. Pollard JC, Littlejohn RP. Behavioral-effects of light conditions on red deer in a holding pen. Applied Animal Behaviour Science. 1994;41(1-2):127-34.

296. Pollard JC, Littlejohn RP. The effects of pen size on the behaviour of farmed red deer stags confined in yards. Applied Animal Behaviour Science. 1996;47(3-4):247-53.

297. Goddard P. Deer Handling and Transport. Grandin T, editor2014. 342-77 p.

298. Grigor PN, Goddard PJ, Littlewood CA. The movement of farmed red deer through raceways. Applied Animal Behaviour Science. 1997;52(1-2):171-8.

299. Grigor PN, Goddard PJ, MacDonald AJ, Brown SN, Fawcett AR, Deakin DW, et al. Effects of the duration of lairage following transportation on the behaviour and physiology of farmed red deer. Veterinary Record. 1997;140(1):8-12.

300. Blackmore DK, Cook CJ, Devine CE, Gilbert KV, Tavener A, Langdon S, et al. Electrical stunning of red deer (Cervus elaphus). New Zealand Veterinary Journal. 1993;41(3):126-30.

301. Cook CJ, Gilbert KV, Devine CE, Dean JM, Hogg B. Minimum duration of effective head-only electrical stunning of fallow deer (Dama dama) and time to loss of consciousness following a throat-cut. New Zealand Veterinary Journal. 1994;42(4):156-7.

302. Cook CJ, Devine CE, Gilbert KV, Jacobson LH, Blackmore DK. Electrical head-only stunning of fallow deer (Dama dama). New Zealand Veterinary Journal. 1994;42(1):38-9.

303. Mulley RC, Falepau DF, Flesch JS, Wiklund E. Rate of blood loss and timing of exsanguination on prevalence of ecchymosis in fallow deer (Dama dama). Meat Science. 2010;85(1):21-5.

304. Lemrhamed A, Tabite R, Farh M, Riad F, El Abbadi N, Tahri EH, et al. Evaluation of preslaughter stress responses during waiting time at lairage in dromedary camels (Camelus dromedarius). Journal of Camel Practice and Research. 2019;26(2):149-56.

305. Hamad B, Hadef L, Aggad H. Effect of lairage time on the physiological and hematological parameters of dromedary camel (Camelus dromedarius). Biological Rhythm Research. 2019.

306. Windschnurer I, Fischer L, Yanagida T, Eibl C, Franz S, Waiblinger S. Caretaker attitudes and animal training are associated with alpaca behaviour towards humans?An online survey. Applied Animal Behaviour Science. 2021;236.

307. Windschnurer I, Eibl C, Franz S, Gilhofer EM, Waiblinger S. Alpaca and llama behaviour during handling and its associations with caretaker attitudes and human-animal contact. Applied Animal Behaviour Science. 2020;226.

308. Biffin TE, Hopkins DL, Bush RD, Hall E, Smith MA. The effects of season and post-transport rest on alpaca (Vicunga pacos) meat quality. Meat Science. 2020;159.

309. Fowler ME, Bravo PW, Fowler ME. Restraint and Handling2010. 59-87 p.

310. Waiblinger S, Hajek F, Lambacher B, Wittek T. Effects of the method of restraint for shearing on behaviour and heart rate variability in alpacas. Applied Animal Behaviour Science. 2020;223.

311. Gibson TJ, Whitehead C, Taylor R, Sykes O, Chancellor NM, Limon G. Pathophysiology of penetrating captive bolt stunning in Alpacas (Vicugna pacos). Meat Science. 2015;100:227-31.

312. Limon G, Guitian J, Gregory NG. A note on the slaughter of llamas in Bolivia by the puntilla method. Meat Science. 2009;82(3):405-6.

313. Kadim IT, Farouk MM, Mahgoub O, Bekhit AE-D. Slaughtering and Processing of Camels. Kadim IT, Mahgoub O, Faye B, Farouk MM, editors2013. 54-72 p.

314. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, et al. Stunning methods and slaughter of rabbits for human consumption. EFSA Journal. 2020;18(1):e05927-e.

315. European Commission, 2017. Preparation of best practices on the protection of animals at the time of killing. Available online: https://publications.europa.eu/en/publication-detail/-/publication/ea4ef3e9-cda5-11e7-a5d5-

316. European Food Safety Authority. Stunning methods and slaughter of rabbits for human consumption. 2019.

317. European Commission. How to stun/kill rabbits on-farm – Factsheet. 2018.

318. Limon G, Gonzales-Gustavson EA, Gibson TJ. Investigation Into the Humaneness of Slaughter Methods for Guinea Pigs (Cavia porcelus) in the Andean Region. Journal of Applied Animal Welfare Science. 2016;19(3):280-93.

319. Cohen S, Kwok M, Huang J. Humane Euthanasia of Guinea Pigs (Cavia porcellus) with a Penetrating Spring-Loaded Captive Bolt. Animals. 2020;10(8).

320. Llonch P, Rodriguez P, Velarde A, de Lima VA, Dalmau A. Aversion to the inhalation of nitrogen and carbon dioxide mixtures compared to high concentrations of carbon dioxide for stunning rabbits. Animal Welfare. 2012;21:123-9.

321. Dalmau A, Pallisera J, Pedernera C, Munoz I, Carreras R, Casal N, et al. Use of high concentrations of carbon dioxide for stunning rabbits reared for meat production. World Rabbit Science. 2016;24(1):25-37.

322. Walsh JL, Van de Vegte J, Mercer B, Turner PV. Euthanasia of meat rabbits with carbon dioxide: Behavioral and physiologic responses to gas chamber gradual- and fast-fill rates. Can Vet J. 2019;60(7):770-8.

323. Rota Nodari S, Lavazza A, Candotti P. Technical Note: Rabbit welfare during electrical stunning and slaughter at a commercial abattoir. World Rabbit Science. 2010;Vol 17, No 3 (2009).

324. Grilli C, Loschi AR, Rea S, Stocchi R, Leoni L, Conti F. Welfare indicators during broiler slaughtering. British Poultry Science. 2015;56(1):1-5.

325. Warriss PD, Bevis EA, Brown SN, Edwards JE. Longer journeys to processing plants are associated with higher mortality in broiler chickens. British Poultry Science. 1992;33(1):201-6.

326. Voslarova E, Chloupek P, Chloupek J, Bedanova I, Pistekova V, Vecerek V. The effects of chronic intermittent noise exposure on broiler chicken performance. Animal Science Journal. 2011;82(4):601-6.

327. Campo JL, Gil MG, SG. D. Effects of specific noise and music stimuli on stress and fear levels of laying hens of several breeds. Applied Animal Behaviour Science. 2005;91:75-84.

328. Nicol, C.J., Bouwsema, J.*, Caplen, G., Davies, A.C., Hockenhull, J., Lambton, S.L., Lines, J.A.*, Mullan, S., Weeks, C.A. Farmed Bird Welfare Science Review. 2017. Published by the Department of Economic Development, Jobs, Transport and Resources 1 Spring Street, Melbourne, Victoria, 3000

329. Raccoursier M, Thaxton YV, Christensen K, Aldridge DJ, Scanes CG. Light intensity preferences of broiler chickens: implications for welfare. Animal. 2019;13(12):2857-63.

330. Warriss PD, Pagazaurtundua A, Brown SN. Relationship between maximum daily temperature and mortality of broiler chickens during transport and lairage. British Poultry Science. 2005;46(6):647-51.

331. Carvalho RH, Honorato DCB, Guarnieri PD, Soares AL, Pedrão MR, Oba A, et al. Assessment of turkey vehicle container microclimate on transit during summer season conditions. International Journal of Biometeorology. 2018;62(6):961-70.

332. Aldridge DJ, Luthra K, Liang Y, Christensen K, Watkins SE, Scanes CG. Thermal Micro-Environment during Poultry Transportation in South Central United States. Animals. 2019;9(1).

333. Vieira F, Silva I, Barbosa Filho JA, Vieira A, Broom D. Preslaughter mortality of broilers in relation to lairage and season in a subtropical climate. Poultry science. 2011;90:2127-33.

334. Wein Y, Bar Shira E, Friedman A. Avoiding handling-induced stress in poultry: use of uniform parameters to accurately determine physiological stress. Poultry Science. 2017;96(1):65-73.

335. Bassler AW, Arnould C, Butterworth A, Colin L, De Jong IC, Ferrante V, et al. Potential risk factors associated with contact dermatitis, lameness, negative emotional state, and fear of humans in broiler chicken flocks. Poultry Science. 2013;92(11):2811-26.

336. Edwards LE, Coleman GJ, Hemsworth PH. Close human presence reduces avoidance behaviour in commercial caged laying hens to an approaching human. Animal Production Science. 2013;53(12):1276-82.

337. Butterworth, A. Welfare Quality®, Assessment Protocol for Poultry (Broilers, Laying Hens). Welfare Quality® consortium, Lelystad, Netherlands ISBN: 9789078240068. 2009.

338. Wigham E, Grist A, Mullan S, Wotton S, Butterworth A. The Influence of Welfare Training on Bird Welfare and Carcass Quality in Two Commercial Poultry Primary Processing Plants. Animals. 2019;9(8).

339. Tremolada C, Bielińska H, Minero M, Ferrante V, Canali E, Barbieri S. Animal-Based Measures for the On-Farm Welfare Assessment of Geese. Animals. 2020;10(5).

340. Jones RB, Marin RH, Satterlee DG. Adrenocortical responses of Japanese quail to a routine weighing procedure and to tonic immobility induction. Poultry Science. 2005;84(11):1675-7.

341. Human Slaughter Association. Practical Slaughter of Poultry – A Guide for the Smallholder and Small-Scale Producer (2016)

342. Woolcott CR, Torrey S, Turner PV, Chalmers H, Levison LJ, Schwean-Lardner K, et al. Assessing a Method of Mechanical Cervical Dislocation as a Humane Option for On-Farm Killing Using Anesthetized Poults and Young Turkeys. Front Vet Sci. 2018;5:275.

343. Stiewert A, Wooming B, Archer G. Comparing various euthanasia devices and methods on 8 and 12-week-old turkey hens. Poultry Science. 2021;100:101053.

344. Martin JE, Sandilands V, Sparrey J, Baker L, McKeegan DEF. On Farm Evaluation of a Novel Mechanical Cervical Dislocation Device for Poultry. Animals. 2018;8(1).

345. RSPCA. RSPCA Approved arming Scheme Standard - Turkeys. 2020.

346. Jacobs L, Bourassa DV, Harris CE, Buhr RJ. Euthanasia: Manual versus Mechanical Cervical Dislocation for Broilers. Animals. 2019;9(2).

347. Martin JE, Sandercock DA, Sandilands V, Sparrey J, Baker L, Sparks NHC, et al. Welfare Risks of Repeated Application of On-Farm Killing Methods for Poultry. Animals (Basel). 2018;8(3).

348. Martin JE, McKeegan DEF, Sparrey J, Sandilands V. Evaluation of the potential killing performance of novel percussive and cervical dislocation tools in chicken cadavers. British Poultry Science. 2017;58(3):216-23.

349. Bandara RMAS, Torrey S, Turner PV, Schwean-Lardner K, Widowski TM. Anatomical Pathology, Behavioral, and Physiological Responses Induced by Application of Non-penetrating Captive Bolt Devices in Layer Chickens. Frontiers in Veterinary Science. 2019;6(89).

350. Gibson TJ, Rebelo CB, Gowers TA, Chancellor NM. Electroencephalographic assessment of concussive non-penetrative captive bolt stunning of turkeys. British Poultry Science. 2018;59(1):13-20.

351. Gibson TJ, King E, Spence J, Limon G. Pathophysiology of Concussive Non-Penetrative Captive Bolt Stunning of Turkeys. Animals. 2019;9(12).

352. Stiewert AM, Archer GS. Comparing two captive bolt devices on market age Pekin ducks. Journal of Applied Poultry Research. 2021;30(2):100162.

353. Huang JC, Chen KJ, Huang M, Meng WG, Sun XB, Xu BC, et al. Effect of pre-slaughter shackling and wing flapping on plasma parameters, postmortem metabolism, AMPK, and meat quality of broilers. Poultry science. 2018;97(5):1841-7.

354. RSPCA. RSPCA Approved arming Scheme Standard - Layer hens. 2015..

355. Scanes CG, Hurst K, Thaxton Y, Archer GS, Johnson A. Effect of transportation and shackling on plasma concentrations of corticosterone and heterophil to lymphocyte ratios in market weight male turkeys in a commercial operation. Poultry Science. 2020;99(1):546-54.

356. Zulkifli I, Wakiman Z, Sazili AQ, Goh YM, Jalila A, Zunita Z, et al. Effect of shackling, electrical stunning and halal slaughtering method on stress-linked hormones in broilers. South African Journal of Animal Science. 2019;49(3):598-603.

357. Smaldone G, Capezzuto S, Ambrosio RL, Peruzy MF, Marrone R, Peres G, et al. The Influence of Broilers' Body Weight on the Efficiency of Electrical Stunning and Meat Quality under Field Conditions. Animals. 2021;11(5).

358. Humane Slaughter Association. 2015. Electrical Waterbath Stunning of Poultry. Humane Slaughter Association. The Old School . Brewhouse Hill Wheathampstead . Herts AL4 8AN . UK

359. Scientific Opinion on the electrical requirements for waterbath stunning equipment applicable for poultry. EFSA Journal. 2012;10(6).

360. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, et al. Slaughter of animals: poultry. EFSA J. 2019;17(11):e05849.

361. Siqueira TS, Borges TD, Rocha RMM, Figueira PT, Luciano FB, Macedo REF. Effect of electrical stunning frequency and current waveform in poultry welfare and meat quality. Poult Sci. 2017;96(8):2956-64.

362. Mercogliano R, Santonicola S, Murru N, Paciello O, Pagano T, Peruzy M, et al. Study on the effects of electrical stunning parameters for broilers on biochemical and histological markers of stress and meat quality. Animal Production Science. 2017;57(6):1144.

363. Anastasov, MI., & Wotton, SB. Survey of the incidence of post stun behavioural reflexes in electrically stunned broilers in commercial conditions and the relationship of their incidence with the applied water bath electrical parameters. Animal Welfare, 2012;22, 247 .

364. Website.Topkip head-only stunning of poultry - http://www.topkip.com/ [Accessed 11/11/2021]

365. Fuseini A. Electrical water bath stunning for Halal poultry meat production: animal welfare issues and compatibility with the Halal rules. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources. 2018;13(016).

366. Wotton S, Grist A, O'Callaghan M, van Klink E. Head-Only Stunning of Turkeys Part 2: Subjective and Objective Assessment of the Application of AC and DC Waveforms. Animals. 2021;11(2).

367. Article: The pros and cons of controlled atmosphere stunning: Controlled atmosphere stunning is coming to the poultry industry. Poultry International. 2018.

368. Raj ABM. Recent developments in stunning and slaughter of poultry. World's Poultry Science Journal. 2006;62(03):467-84.

369. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, Depner K, Drewe JA, et al. Slaughter of animals: poultry. EFSA Journal. 2019;17(11).

370. Gerritzen MA, Reimert HG, Hindle VA, Verhoeven MT, Veerkamp WB. Multistage carbon dioxide gas stunning of broilers. Poult Sci. 2013;92(1):41-50.

371. Guijarro A, Mauri S, Aviles C, Peña F. Effects of Two CO2 Stunning Methods on the Efficacy of Stunning and Blood Stress Indicators of Turkeys under Commercial Processing Conditions. Journal of Applied Animal Welfare Science. 2020;23(2):231-43.

372. Nielsen SS, Alvarez J, Bicout DJ, Calistri P, et al. Killing for purposes other than slaughter: poultry. EFSA J. 2019;17(11):e05850.

373. Holloway PH, Pritchard DG. Effects of ambient temperature and water vapor on chamber pressure and oxygen level during low atmospheric pressure stunning of poultry. Poult Sci. 2017;96(8):2528-39.

374. Gent TC, Gebhardt-Henrich S, Schild S-LA, Rahman AA, Toscano MJ. Evaluation of Poultry Stunning with Low Atmospheric Pressure, Carbon Dioxide or Nitrogen Using a Single Aversion Testing Paradigm. Animals. 2020;10(8).

375. Martin JE, McKeegan DEF, Magee DL, Armour N, Pritchard DG. Pathological consequences of low atmospheric pressure stunning in broiler chickens. Animal. 2020;14(1):129-37.

376. Humane Slaughter Association. Effective neck-cutting of poultry. Humane Slaughter Association. The Old School . Brewhouse Hill Wheathampstead . Herts AL4 8AN . UK

377. Animal Health Australia. Operational manual: Destruction of animals (Version 3.2). Australian Veterinary Emergency Plan (AUSVETPLAN), Edition 3, Agriculture Ministers' Forum, Canberra, ACT2015.

378. Minka NS, Ayo JO. Assessment of the stresses imposed on adult ostriches (Struthio camelus) during handling, loading, transportation and unloading. Veterinary Record. 2008;162(26):846-51.

379. Wotton S, Sparrey J. Stunning and slaughter of ostriches. Meat science. 2002;60:389-94.

380. Model code of practice for the welfare of animals: husbandry of captive-bred emus. CSIRO Publishing. 2006

381. Model code of practice for the welfare of animals: farming of ostriches. CSIRO Publishing. 2003.

382. Hoffman LC. Advances in the electrical stunning and bleeding of ostriches. Animal Welfare. 2012;21:9-13.

383. Crowther C, Davies R, Glass W. The effect of night transportation on the heart rate and skin temperature of ostriches during real transportation. Meat Science. 2003;64(4):365