
Quantitative assessment of the welfare impacts of methods for the commercial slaughter of broilers

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I. Background

On any day, over 200 million chickens are slaughtered for food, a figure that excludes unproductive laying hens killed in the egg production chain and newly born male chicks deemed unsuitable for meat production. It amounts to over 2.3 thousand chickens killed per second, representing more than 95% of all land animals slaughtered for food on this planet [1]. A sense of scale is provided in Figure 1.



Figure 1. Number of chickens slaughtered per year (2019, FAOSTAT) compared to other land animals and the global human population.

With millions of animals slaughtered daily, even small differences in the magnitude of the pain associated with the different manners of death that befall these birds become of importance to inform strategies to reduce suffering in the poultry industry. In this study, we quantify the welfare impacts of different slaughter methods used for poultry.

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Although originally developed as a means of immobilising the animal for ease of processing [2], recognition that the pain endured during slaughter should be alleviated has become the main driver for the implementation of slaughter methods that include an initial stunning stage to render the animal unconscious and insensible to pain — ideally in an immediate and painless way [3] — until the time of death [2]. For instance, since 1974 European legislation requires that all animals to be killed for human consumption must be stunned before they are slaughtered (Council Directive 74/577/EEC). Similarly, most countries in Latin America have some legislation in place that makes it compulsory to use methods to stun animals prior to their slaughter for consumption [4].

Unfortunately, pre-slaughter stunning regulation is still lacking in many parts of the world [2] and, where present, enforcement is poor and legal loopholes abound [5]. For example, European legislation on welfare at the time of killing states that animals “*must be spared any avoidable pain, distress or suffering during their killing*” [6]. Yet, the choice of the term ‘avoidable’ in several paragraphs of the regulation leaves room for interpretation and flexibility in the implementation. In the United States, poultry are ‘not’ listed under the Humane Methods of Slaughter Act, hence there is no legal requirement for pre-slaughter stunning — its practice is merely voluntary [7,8]. The observed frailties in the legislation in Europe and the US are an indicator of welfare during slaughter in other regions.

Even where pre-slaughter stunning is widely implemented, stunning methods will differ in their effectiveness and welfare impacts depending on their inherent characteristics and the extent to which they are properly implemented. Existing methods mostly fall in three categories: mechanical, electrical, and controlled atmosphere stunning (CAS). While mechanical stunning (e.g., cervical dislocation) is predominantly used at small-scale or as a back-up, electrical and, to a lesser extent, CAS stunning are the methods of choice in commercial slaughterhouses [9].

In this chapter we quantify the welfare impacts of existing and potential policies on the stunning of poultry prior to slaughter. To this end, we use the [Cumulative Pain Framework](#) [10] to estimate the loss of welfare endured by birds exposed to electrical waterbath stunning, the most commonly used method for poultry stunning globally [16,17], and multi-stage controlled atmosphere stunning with CO₂, the most commonly applied stunning gas under commercial conditions.

II. Pre-Stunning Stages

Upon arrival at the slaughter plants, transport trucks may be directed to a waiting area which, depending on the region, may be acclimatized (e.g. in warmer regions the use of fans and water misting is common). Alternatively, bird crates may be unloaded from trucks, usually with forklifts. Automated unloading systems have also been developed where crate modules are lifted and tilted directly onto a conveyor belt. Based on our personal observations (ENC), truck unloading tends to be relatively swift once starting, lasting from 5 to 15 minutes.

Once unloading is complete, the birds may be placed onto conveyor belts that take them directly to the slaughter line, or they may be held at the lairage, a waiting area aimed at reducing thermal stress resulting from transport and keeping a stock of poultry to ensure the slaughterhouse can operate at a constant speed and not be affected by transport delays. Lairage time is variable, depending on the throughput rate and organization of the slaughterhouse. For broilers, waiting times can range from practically no time up to nearly 18 hours [11,12].

Next, it may then take approximately 5-10 minutes for the crates to be transported from the lairage to the stunning point (*personal observation*, ENC).

III. Electrical Waterbath Stunning

Electrical stunning is based on passing an electrical current through the brain which, if properly delivered, should cause depolarization of neurons of both cerebral hemispheres and epileptiform activity. At this state, neurons cannot properly generate or transmit electrical impulses and brain functioning is temporarily impaired. Loss of consciousness and insensitivity to pain will be induced for a period of time (shorter than a minute; [13]), until the neurons are able to repolarize again, resuming normal brain activity [14,15].

Electrical stunning can be implemented in three ways, depending on how the electric current is administered: (1) by dipping the birds into an electrical waterbath; (2) by applying current individually to each bird on either side of the head through two electrodes (head only stunning); and (3) by applying current first to the head and afterward through a second electrode placed on the cloaca (head-to-cloaca stunning). In this section we describe and discuss the waterbath method only, as the other ones are not yet fully commercialized.

Briefly, waterbath stunning involves manually catching chickens from their crates and inverting them upside down, then hanging them by the shanks of both legs into parallel metal slots of an assembly line [16] (Figure 2). This is done in a single movement and takes a few seconds for each bird shackled. Catching, inverting, and shackling birds by the shanks causes various types of lesions, pain and fear [11]. This is exacerbated in birds with leg abnormalities and/or with recent fractures endured during the pre-slaughter stages (i.e. during depopulation at the farm, truck loading, transport, and unloading) [17] and in larger sized birds, which will have their legs forced into inadequate shackles for their leg conformation [18]. The longer the birds are shackled, the higher the levels of stress reached [19].

Once birds are shackled, the duration of subsequent phases depends largely on the speed and design of the slaughter line. For reference, broiler line speeds in Europe can reach up to 216 birds per minute [20]. In the US, line speeds are regulated to a maximum of 140 birds/min, except for plants that were issued temporary waivers in 2020 to operate at a line speed of up to 175 birds per minute [21,22]. Fast line speeds have been shown to have a substantial negative impact on the welfare of both birds and workers [23,24], leading to exhaustion and the inappropriate handling of birds [11].

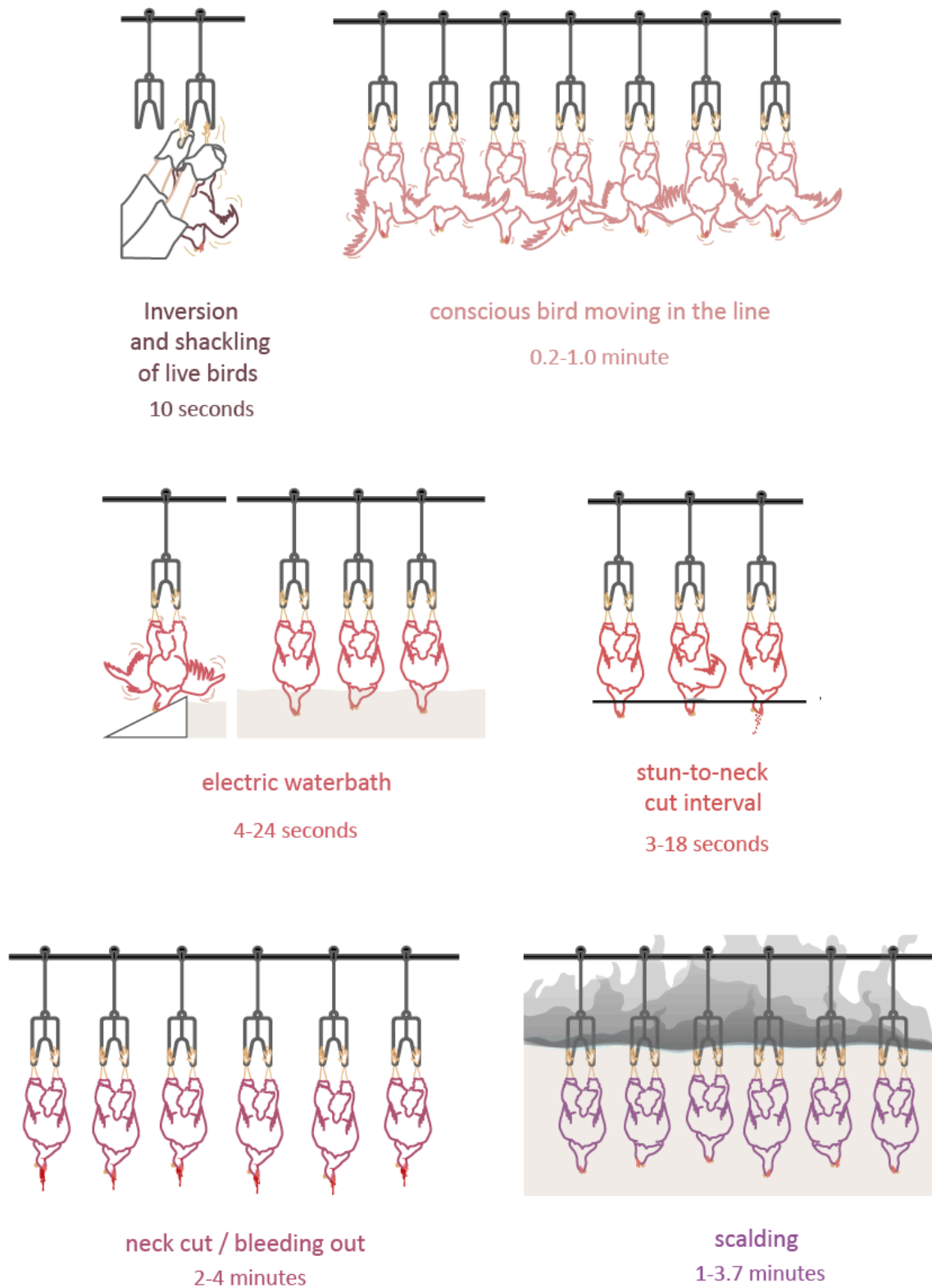


Figure 2. Electrical waterbath stunning phases and estimated durations . The scalding phase has been added since some birds reach the scalding tank still conscious. Sources for duration: electric waterbath [11], stun-to-neck cut interval [11,25], neck cut/bleeding out [26,27], scalding [28]. Illustration: Robson Rezende.

Subsequent to shackling, birds reach the waterbath device in a few seconds [11] (Fig.2). In general, the time between hanging and stunning should be no longer than 30 seconds to prevent the blood from depositing at the wings due to gravity [29]. At the entrance of the waterbath, a smooth entry ramp with a steep decline should be present, electrically isolated [18], and adjusted to the size of the birds, so the bird's head slides upwards onto the ramp. Once it reaches the end of the ramp, the head will swing abruptly into the electrified water [30]. An electric potential difference is generated in the waterbath by means of a submerged electrode, across a circuit between the electrified water, the body of the bird, the metal shackles, and the earthed rubbing bar. When the head of the bird enters the water, the circuit is completed. The minimum time required at the waterbath is 4 seconds [11]. If the appropriate current is administered, epileptiform activity in the brain will be induced, rendering the animal unconscious [9,16].

Multiple variables, such as the size and characteristics of the bird (e.g. bone density, weight), the design and speed of the slaughter line, and features of the electrical stunning device will influence the extent to which stunning is effective [31]. Bird behavior can also impact the process: attempts by the birds to right themselves, or too much movement will prevent the birds' heads from reaching the water. Similarly, the birds' reaction to the electric shock, by moving their heads up, may prevent the mechanism from functioning as designed. The quality of the equipment and stewardship of the process is equally important: if the water level is not constantly monitored, water may overflow through the ramp, causing one or several pre-stun shocks [11,32]. Conversely, if the water level becomes too low (e.g. due to the constant absorption of some of the water by passing heads), the heads will not be sufficiently submerged into the electrified water, rendering stunning ineffective.

The combination of electrical parameters used (current, voltage, frequency, current type and waveform) is also critical to determine whether stunning renders the birds unconscious, immobile or dead [13,31] (Figure 3). If the current is too low and frequency is high, it can cause electro-immobilization [18], rendering the animal immobile but fully conscious. Even with appropriate current, if several birds are immersed in the waterbath, individuals with a higher electrical resistance (e.g. due to body composition, size, feather cover) may receive insufficient current to be adequately stunned. Most of the electrical resistance is attributed to poor contact between the legs and the shackle (tighter leg-shackle fitting will offer better chances of effective stunning, but at the expense of more pain during shackling [33]). It is indeed widely acknowledged that it is impossible to deliver the same constant current to each individual bird [11,13,33]. The amperage received might also be insufficient, causing birds to start moving within seconds. Contamination of the water with feathers, blood and other material may also affect its conductivity, reducing stunning effectiveness.

The EU Regulation 1099/2009 sets out a wide range of parameters for electrical stunning, including currents of at least 100-200 mA, linked to frequencies from <200 to 1500 Hz [6]. These parameters are compatible with variable rates of stunning effectiveness. A minimum current of 120 mA has been recommended, which, if supplied with a 50 Hz sine wave alternating current, would stun-kill 90% of the birds [3,34]. An increase to 148mA has been associated with the stun-kill of 99% of the birds [34]. Stun-kill occurs when the electrical frequency is such that the heart muscles are stimulated to the point of ventricular fibrillation, causing death by cardiac arrest [16,35]. From a welfare perspective, stun-kill tends to be favoured as it removes the risk of birds regaining consciousness during or after neck-cutting.

However, cardiac ventricular fibrillation is not necessarily accompanied by prior induction of epileptiform activity in the brain (unconsciousness), and consequent insensitivity to pain [34]. Although a few EU Member States (e.g. Sweden) have adopted higher standards of welfare

compared to those set out in Regulation 1099/2009 [3], the specific parameters increasing the effectiveness of stunning are not legally mandated, and no requirement is made regarding the type of current (pulsed direct current versus the more effective sine wave alternating current [36]). In fact, some of the frequencies currently permitted (>800 Hz) might lead to electro-immobilization only [13]. This situation is exacerbated in non-EU countries that lack legislation on stunning parameters.

The use of high voltage and low frequency, associated with a higher stunning effectiveness, also lead to carcass damage [37], as lower-frequency shocks will trigger more intense muscle seizures, increasing the likelihood of hemorrhages, broken bones, damaged viscera, and bruises [3,37]. Therefore, companies may favor the use of low voltage to ensure minimal carcass damage, despite this resulting only in the immobilization of birds, without loss of consciousness. This is the case of the United States, where most poultry slaughterhouses are reported to use low voltage systems with high frequency (>300 Hz) [38,39]. In these systems, bleeding must be performed very quickly (within about 10 seconds), as in 30-60 seconds birds may regain consciousness [40]. In general, the higher the frequency, the faster the recovery of unconsciousness — with frequencies higher than 800Hz, animals are likely to regain consciousness before bleeding [40].

Soon after the birds exit the electrical waterbath, their carotid arteries are cut by an automatic neck-cutter blade. If they were not stun-killed before, death should be caused by exsanguination (blood loss). However, birds who were not properly stunned may raise their heads, avoiding the blade. Or the blade may not be effective for all bird sizes and conformations. Although staff should check that all birds are unconscious when they leave the stunner, in practice this is very difficult to achieve both because of the fast speed of the line and because reflexes, muscle tone and direct observations are not reliable indicators of unconsciousness [13].

Thus, large numbers of birds are still conscious when they reach the scalding tank [11]. For example, based only on official records in federally inspected slaughter plants in the United States [41], over 500 thousand chickens were scalded alive in that country in 2019 (though this figure is likely underestimated). In an independent study in Dutch abattoirs, the authors observed that an average of 5.4% of birds had not been properly stunned at the entrance of the scalding tank [42]. At a global scale, the extent of the problem is likely vast as, similar to the United States, most countries lack regulations on stunning parameters.

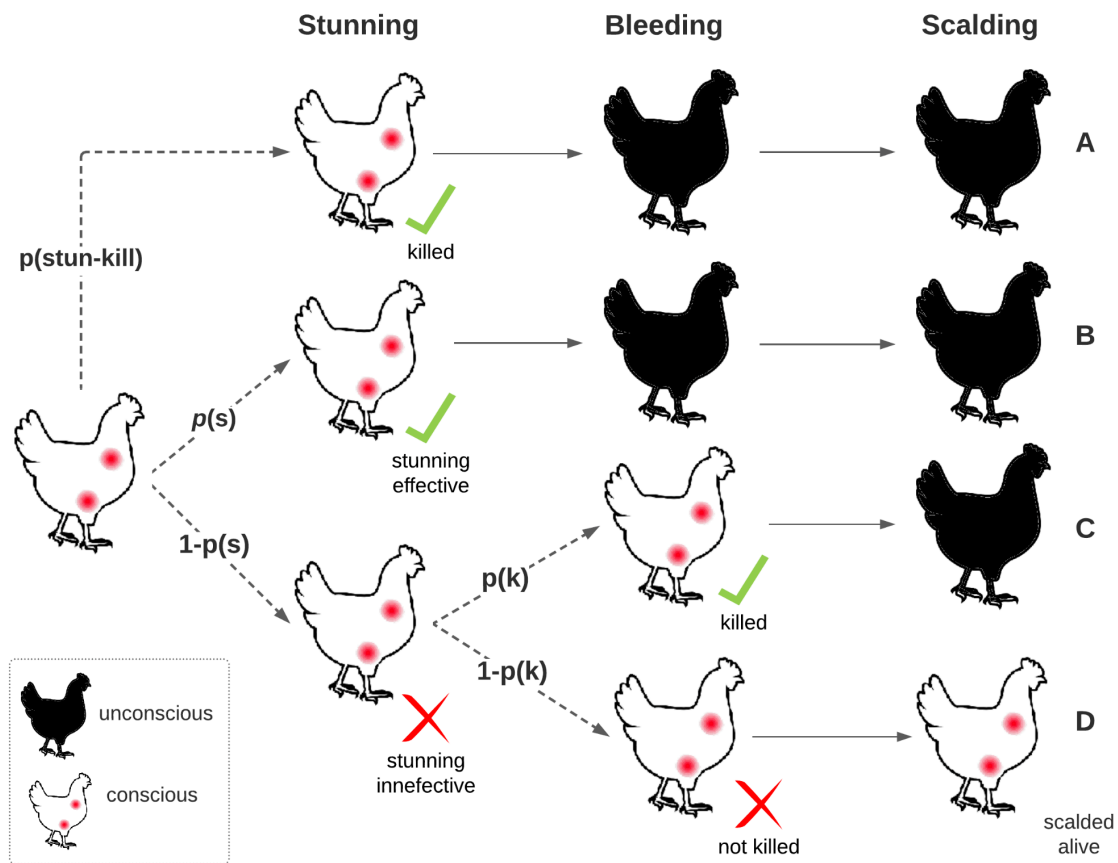


Figure 3. Possible outcomes during the stunning process, until scalding. Birds may experience one of four “fates” (A to D), depending on $p(s)$, the probability of effective stunning (birds may not be stunned or may regain consciousness), and $p(k)$, the probability that conscious birds are killed at the bleeding phase. Birds can experience pain (represented by colored red symbols within the bird outline) only when conscious. Black birds represent unconscious or dead individuals. Birds stun-killed are represented by a different fate (A). The flow chart applies both to electrical waterbath systems and controlled atmosphere stunning.

Figure 3 summarizes the possible outcomes experienced by birds in each phase of the stunning process, as based on the effectiveness of the procedure to induce loss of consciousness. As suggested by a review of the European Food Standards Agency [13], although the aim of a stunning system is to achieve a 100% effective stun, the most effective electrical parameters can achieve an effectiveness of up to 96% as measured using EEG ($p(s)$). Therefore, we assume that **4-10% of chickens will be conscious before bleeding ($1-p(s)$ in Figure 3) should an electrical waterbath system be ideally implemented.** Based on the work of de Jong and collaborators [42], and a review on the effectiveness of stunning with different parameters [13], we tentatively estimate that these figures ($1-p(s)$) may typically vary from 5 to 70% of birds in the United States and other countries lacking regulation on stunning parameters. The possibility that up to 30% of the birds are still conscious after leaving the stunner is based on the expectation that, if parameters are set to ensure minimal carcass damage (a priority for the industry), a large fraction of birds may suffer electro-immobilization without loss of consciousness. **The interval is, however, purportedly wide given the great degree of uncertainty on these parameters.** Birds not stunned effectively are those for which the parameters do not induce loss of consciousness as well as those that regain consciousness before bleeding.

We also estimate that **0.01% to 6% of birds may reach the scalding tank still conscious in countries lacking regulation on stunning parameters**. The upper limit is based on an independent study that measured this parameter for flocks from 10 farms in a Dutch abattoir *before European regulations on stunning were implemented* [42], and which reports $5.4 \pm 1.4\%$ of birds entering the scalding tank still conscious. This upper limit is possibly conservative, as measurements were conducted in slaughter plants that had agreed with the inspections and installation of video cameras at several points of the slaughter line (hence less likely to incur into errors and neglect issues). The lower limit reflects the possibility that the percentage is much lower, as reported by the USDA for officially inspected plants [41].

Considering the larger proportion of birds effectively stunned where stunning parameters are all properly set and regulated, **we assume that the number of birds reaching the scalding tank still conscious is ten times lower (0.001 to 0.6%) in such systems**. Given the large uncertainty regarding these parameters, we assume any value within these ranges is equally likely (i.e. an uniform distribution). Further studies in abattoirs, particularly in major chicken producing countries, are very much needed to determine these figures more accurately.

In the next sections we use the [Cumulative Pain Framework](#) [10] to evaluate the welfare impacts of waterbath stunning. Specifically, we focus on the likely time spent in four categories of increasing pain intensity (Annoying, Hurtful, Disabling and Excruciating) as a result of the physical pain resulting from (1) inversion and live shackling (respiratory distress, wing fractures and leg pain), (2) the electrical shocks at the waterbath stunner, (3) the pain associated with neck cutting and bleeding in conscious birds and, (4) the pain associated with live scalding and (5) the psychological pain from fear. All experiences are referred to simply as ‘pain’, which in this framework is defined as ‘any felt negative affective state’.

Box 1 Definition of Pain Intensity Categories

Annoying: experiences of pain perceived as aversive, but not intense enough to disrupt the animal’s routine in a way that alters adaptive functioning or affects the behaviors that animals are motivated to perform. Similarly, Annoying pain should not deter individuals from enjoying pleasant experiences with no short-term function (e.g., play) and positive social interactions. Sufferers can ignore this sensation most of the time. Performance of cognitive tasks demanding attention are either not affected or only mildly affected. Physiological departures from expected baseline values are not expected to be present. Vocalizations and other overt expressions of pain should not be observed.

Hurtful: experiences in this category disrupt the ability of individuals to function optimally. Different from Annoying pain, the ability to draw attention away from the sensation of pain is reduced: awareness of pain is likely to be present most of the time, interspersed by brief periods during which pain can be ignored depending on the level of distraction provided by other activities. Individuals can still conduct routine activities that are important in the short-term (e.g. eating, foraging) and perform cognitively demanding tasks, but an impairment in their ability or motivation to do so is likely to be observed. Although animals may still engage in behaviors they are strongly motivated to perform (i.e., exploratory, comfort, sexual, and maintenance behaviors), their frequency or duration is likely to be reduced. Engagement in positive activities with no immediate benefits (e.g., play in piglets, dustbathing in chickens) is not expected. Reduced alertness and inattention to ongoing stimuli may be present. The effect of (effective) drugs (e.g., analgesics if pain is physical, psychotropic drugs in the case of psychological pain) in the alleviation of symptoms is expected.

Disabling: pain at this level takes priority over most bids for behavioral execution, and prevents all forms of enjoyment or positive welfare. Pain is continuously distressing. Individuals affected by harms in this category often change their activity levels drastically (the degree of disruption in the ability of an organism to function optimally should not be confused with the overt expression of pain behaviors, which is less likely in prey species). Inattention and unresponsiveness to milder forms of pain or other ongoing stimuli and surroundings is likely to be observed. Relief often requires higher drug dosages or more powerful drugs. The term Disabling refers to the disability caused by 'pain', not to any structural disability.

Excruciating: all conditions and events associated with extreme levels of pain that would not normally be tolerated even if only for a few seconds. In humans, it would mark the threshold of pain under which many people choose to take their life rather than endure the pain. This is the case, for example, of scalding and severe burning events. Behavioral patterns associated with experiences in this category may include loud screaming, involuntary shaking, extreme muscle tension or extreme restlessness. Another criterion is the manifestation of behaviors that individuals would strongly refrain from displaying under normal circumstances, as they threaten body integrity (e.g. running into hazardous areas or exposing oneself to sources of danger, such as predators, as a result of pain or of attempts to alleviate it). The attribution of conditions to this level must therefore be done cautiously. Concealment of pain is not possible.

III.1 Pain during Shackling and Inversion

Leg Compression

The shackling process (i.e., catching, inverting and manually inserting a bird's legs into the moving parallel metal slots of the assembly line; Figure 2) must be accomplished swiftly to match the fast speed of the shackle line. The grip must be tight enough to prevent the bird from escaping, ensure proper electrical contact during stunning and avoid dislodging during plucking [43]. Thus, staff must apply forces of five or ten times the birds' weight (around 180 Newtons) to push their legs into the shackles, resulting in their severe compression [43,44].

The tissues compressed by the metal slots - skin, muscles, connective tissues, joints and bones - are not only not prepared for holding the weight of the animal against such a small hard area of the shackle, but they are also highly innervated with nociceptors that have response thresholds much lower than the forces exerted on the leg during shackling [45,46]. Based on observed discharge rates of nociceptors in the legs of chicken in response to mechanical forces, and the association between discharge rates and pain in humans, some authors have concluded that *'significant pain is likely to be inflicted on the animals during shackling'* [46]. Bruising of the leg skin and thigh muscles is typically observed during post-mortem inspection [45].

Irregular movements of the shackle line due to curves and sharp transitions will increase the compression exerted on the sensitive tissues of the legs. By leading to exhaustion of workers and the resulting rough or improper handling of birds, fast line speeds may also increase the proportion of birds that are shackled by just one leg, or even on two different adjacent shackles. Given the uneven distribution of weight in the shackles in these cases, pain is possibly more intense [11,23].

Additionally, although the size and design of the metal shackles is usually standard, the birds' legs' sizes are not. So even when healthy birds are properly handled, the shackles might simply not be fit for purpose, as they might be too narrow or too wide for the birds' shanks anatomy.

Heavier birds (e.g. males) with significantly thicker shanks [47] will require more force to be inserted into the shackles and will be subjected to more compression. Indeed, an earlier onset and a longer duration of behavioural indicators of pain have been observed in heavier birds after shackling [47].

Based on these observations, we estimate that in healthy birds without leg lesions (Pain-Track 1) the intensity of the pain caused by compression in the shackles will be at least of a Hurtful nature, as this is a sensation unlikely to be ignored (Box 1), with only a fraction of birds (tentatively estimated at 30%, representing heavier/male birds and/or those not appropriately shackled) experiencing Disabling pain. More accurate data to adjust this estimate is needed. Durations are those shown in Figure 2.

In birds with leg abnormalities, lameness, or thicker shanks (Pain-Track 2), pain will be likely more intense, as it will be harder to insert the legs into the shackles and more force will be required [11,48,49]. In Chapter 2, we estimate that about 15-45% of fast-growing broilers are affected by moderate and severe lameness (gait scores 3 or higher). In this case, we tentatively assume that the likelihood of Disabling pain is higher.

In injured birds (i.e., dislocated joints, recent bone fractures endured during depopulation or transport), pain will be most likely exacerbated [46], particularly if nociceptors become hyperalgesic due to inflammation [50]. Therefore, in Pain-Track 3 we propose that the intensity of the physical pain endured by injured birds during shackling is predominantly Disabling (Box 1). Based on previous studies reporting the prevalence of broiler chickens with leg fractures and severe lesions endured during depopulation, transport and at the lairage [17,51–54], we estimate that 0.2-4% of birds fall into this category.

Pain-Track 1. Hypothesis for the pain associated with **leg compression during the shackling process in broilers properly handled and with no leg lesions**. The table on the right shows the estimated time in pain (seconds) as a result of this experience.

	shackling	TIME IN PAIN (sec)
Excruciating		
Disabling	30%	16.50 [6-27]
Hurtful	70%	38.5 [14-63]
Annoying		
Duration	20-90 s	

Pain-Track 2. Hypothesis for the pain associated with **leg compression during the shackling process in birds with lameness-causing conditions or heavier**. The table on the right shows the estimated time in pain (seconds) as a result of this experience. Prevalence: 15-45% birds (GS3+ birds, Chapter 2).

	shackling	TIME IN PAIN (sec)
Excruciating		
Disabling	50%	27.50 [10-45]
Hurtful	50%	27.50 [10-45]
Annoying		
Duration	20-90 s	

Pain-Track 3. Hypothesis for the pain associated with **leg compression during the shackling process in injured birds (with fresh fractures and dislocations)**. The table on the right shows the estimated time in pain (seconds) as a result of this experience. Prevalence: 0.2-4% of birds.

	shackling	TIME IN PAIN (sec)
Excruciating		
Disabling	70%	38.5 [14-63]
Hurtful	30%	16.50 [6-27]
Annoying		
Duration	20-90 s	

Inversion

Shackling involves inverting and suspending the bird upside down, an abnormal posture for chickens. It has been hypothesized that since birds do not have diaphragms, inversion might cause the viscera to compress the thorax, compromising pulmonary and cardiac activity, and causing pain and fear [11]. However, for some species of psittacidae and passeriformes, hanging upside down is a natural behaviour. Psittacidae hang upside down to relax or just for fun [55,56], while passeriformes do so when foraging for insects and seeds [57,58]. Thus, as all birds lack diaphragms, it is also possible that lung and heart compression caused by inversion is not as distressful. Given lack of evidence on the distress produced in chickens, we consider that the discomfort associated with the experience of inversion is predominantly Annoying (Box 1). Still, considering the differences in conformation between broiler chickens with heavy breast muscles and other birds, we consider a 20% likelihood that the intensity of the distress from inversion is Hurtful.

Pain-Track 4. Hypothesis for the pain associated with inversion (from shackling until loss of consciousness, assumed to occur in the stunner) The table on the right shows the estimated time in pain (seconds) as a result of this experience.

	Inversion	TIME IN PAIN (sec)
Excruciating		
Disabling		
Hurtful	20%	11 [4-18]
Annoying	80%	44 [16-72]
Duration	20-90 s	

Wing Fractures

Immediately after shackling, 90% of birds will struggle and flap their wings, and most will do so when passing through any unevenness in the line [59]. This is a natural response to losing balance [59]. In some cases, however, this vigorous flapping can cause wing fractures and haemorrhages of the wing tip [31,60]. A study in 12 abattoirs found that 2.9% of the birds suffer wing fractures right after shackling and prior to stunning [54], a prevalence higher than that of wing fractures endured during depopulation and transport [54]. In fact, the Animal Welfare

Guidelines for broilers of the National Chicken Council (USA) states that corrective action must be initiated only if the level of broken or dislocated wings before or after the stunner exceeds 4%.

The immediate pain following a fracture is mainly attributed to activation of mechanosensitive nociceptors in the affected bone and in the surrounding tissues [61,62]. During this initial phase bone fractures are reported as severely painful in humans [62]. In mammals, the periosteum has an extremely dense sensory and sympathetic innervation, in which most of the mechanosensory fibers activated immediately after fracture are believed to be located [63]. This pain-generating process can be reactivated by movements or mechanical distortions of the broken bone, which supports the mechanosensitivity role of the nerve fibers innervating the bone [64]. After the injury, an influx of inflammatory cells to the fracture site results in the release of inflammatory mediators that will cause the swelling of the site (hence further pain through activation of pressor receptors) and activate undamaged nociceptors [61,62,65].

As in mammals, it is adaptive for birds to experience intense pain during the initial, inflammatory phase of a severe fracture: it ensures the reduction of movement needed to facilitate healing and protect the individual from further injury. Given the similarities between avian and mammalian nociceptors [50,66,67] we suggest that broilers experience acute and intense pain immediately following a bone fracture. However, different from typical bone fractures (where the acute pain following a fracture is estimated to be Disabling), we also assign a low (5%) probability to the Excruciating category, given the circumstances in which these fractures occur, including the rough handling of the birds by workers and their shackling and inversion on a moving line.

Pain-Track 5. Hypothesis for the pain associated with **wing fractures during the shackling process**. The table on the right shows the estimated time in pain (seconds) as a result of this experience. Estimated prevalence (see text): 2-5% of birds.

	wing fractures	TIME IN PAIN (seconds)
Excruciating	5%	2.75 [1-4.5]
Disabling	95%	52.25 [19-85.5]
Hurtful		
Annoying		
Duration	20-90 s	

III. 2 Pain during Electrical Waterbath Stunning

To estimate the time in pain experienced during the stunning procedure, we consider four (non-mutually exclusive) possible outcomes, as follows: (i) pre-stun shocks (Pain-Track 6); (ii) stunning is ineffective, with immediate immobilization achieved, but not unconsciousness (Pain-Track 7; Figs.3 C, D) (iii) stunning is effective in producing unconsciousness (electronarcosis) (Pain-Track 8; Fig.3B); and (iv) upon contact with the electrified water, birds are electrocuted and killed (stun-kill) (Pain-Track 9; Fig.3A).

Pre-stun shocks (unconsciousness not immediately achieved)

In humans, when a current above 10 mA travels through extensor muscles, it causes a violent spasm, with muscles, ligaments and tendons often tearing as a result of the sudden contraction [68]. The observation of multisystem injuries following the shocks [69] indicates that, even though there is typically only one point of contact with the electric current, pain is likely experienced in several body parts.

Accordingly, pre-stun shocks have been demonstrated to significantly affect carcass and meat quality in poultry [70,71]. On contact with the electrified water, the current that flows through the bird induces tonic seizures, characterized by the abrupt and vigorous contraction of all body muscles, which will often lead to tissue injury.

The pain associated with electric shocks is expected to have two origins: the pain associated with the activation of nerves near the point of contact and the violent jerk of the muscle contractions [72], followed by the pain associated with the resulting injuries. In broilers, we assume that the pain associated with pre-stun shocks is of a Disabling nature during the shock, as it is a sensation that cannot be ignored and prevents all other forms of behaviour (Box 1). Since birds can move the body part (e.g. a wing) away from the electrified water immediately after receiving the shock, we estimate that each contact with the waterline lasts up to 1 second.

We also assume that pain subsides afterwards, until a second shock is received or consciousness is lost. Given the uncertainty regarding the intensity of the pain right after the shock, we equally distribute the probabilities between the Hurtful and Disabling categories.

To our knowledge, no data is available on the proportion of birds that typically receive pre-stun shocks, or the number of shocks typically received by them. In turkeys, a study conducted two decades ago estimated that 80% of individuals receive pre-stun shocks [73]. However, turkeys are more prone to pre-stun shocks than broiler chickens because their wings hang lower than their heads. **Therefore, we tentatively assume that 10 to 40% of broiler chickens receive one to two shocks in the stunner².** Data for refining this estimate is, once more, very much needed.

Pain-Track 6. Hypothesis for the pain associated with each pre-stun shock prior to loss of consciousness. The table on the right shows the estimated time in pain as a result of each shock. Proportion of birds affected: 10-40%.

	Pre-stun shocks	Immediate Post-shock pain	TIME IN PAIN (sec)
Excruciating			
Disabling	100%	50%	2.44 [1.32-3.56]
Hurtful		50%	1.50 [0.71-2.29]
Annoying			
	0.25 - 1 s	1-3 s	

² Note that analytical outcomes are similar if, say, 2 to 8% of birds receive 5 to 10 shocks, or 4 to 16% of birds receive 2.5 to 5 shocks

Waterbath stunning that leads to immobilization only (conscious immobilization)

Electrical immobilisation rather than unconsciousness is associated with the use of a low current setting with a high frequency, pulsed, direct current (typically 25 – 30 V, 10–45 mA per bird, at 350-500 Hz). Since low current parameters are typically not sufficient to induce epileptiform activity, it is generally assumed that the bird remains conscious while experiencing the shock [11,74]. As put forward by the European Food Safety Agency, “*Clearly, induction of seizures in conscious birds or electro-immobilisation with ineffective stunning current parameters would obviously cause pain and fear*” [11].

High frequency and low current parameters are likely used in many countries outside Europe, as these are best to prevent the tonic muscle contractions and rupture of small blood vessels and other tissues [75], thus ensuring greater meat quality.

We tentatively assume that the pain experienced as a result of a high frequency electrocution for a long time during immersion in the waterbath is at least Disabling, with a similar likelihood of Excruciating pain (Pain-Track 7). The possibility of Excruciating pain is based on reports from victims of electrocution, who will often equate the experience with extreme pain (e.g. “*the electric shocks were the worst form of torture... It felt like my entire body was being pierced with millions of needles*” [76]). Indeed, electrical shocks have been widely used as a form of torture.

Pain-Track 7. Hypothesis for the pain associated with a constant shock in the head (the bird’s head is submerged, but the electrical parameters are not sufficient to induce stunning). The table on the right shows the estimated time in pain as a result of this experience.

	constant shock in the head while conscious (head submerged)	TIME IN PAIN (sec)
Excruciating	50%	7 [2-12]
Disabling	50%	7 [2-12]
Hurtful		
Annoying		
4-24 s		

Waterbath stunning that achieves unconsciousness (electronarcosis)

Achieving an electrical current strong enough to induce immediate loss of consciousness in all birds, rather than immobilization only, is extremely challenging but still possible depending on the parameters applied. Electronarcosis occurs when an electrical current induces an epileptiform state followed by a flat or isoelectric phase, as shown in electroencephalograms (EEGs). This is a clear indicator that the stunning has been effective and that the animal is insensible to pain [38]. A similar mode of action occurs with electro anaesthesia, which has been demonstrated in animal surgery [77].

There is always a delay between the time electricity starts flowing through the head, after contact with the waterline, and the onset of unconsciousness (i.e., stunning is never immediate). During that period, the bird will experience the pain associated with the electric shock in the head. This delay might last up to two seconds. We assume that the pain

experienced in this type of stunning is equivalent to that produced by shocks leading to immobilization only (Pain-Track 6), though in this case duration is substantially shorter.

Pain-Track 8. Hypothesis for the pain associated with stunning parameters that induce unconsciousness. The table on the right shows the estimated time in pain as a result of this experience.

	brief shock in the head	TIME IN PAIN (s)
Excruciating	50%	0.75 [0.5-1]
Disabling	50%	0.75 [0.5-1]
Hurtful		
Annoying		
1-2 s		

Waterbath stunning that electrocutes and kills the bird (stun-kill)

Low frequency and high voltage parameters can induce muscle contractions, ventricular fibrillation, cardiac arrest and ultimately death [78].

In humans, fatal electrocution accidents are often described as causing an extremely rapid death, although some authors claim that death by electrocution could be due to asphyxia caused by paralysis of respiration, in which case several seconds could elapse during which the victim could still be conscious [79]. Similarly to stun-killed birds, during execution by electrocution the violent movement of the limbs resulting from the muscle contractions also produce dislocations and/or fractures.

Although it is not possible to determine the extent to which the subjective experiences of mammals and birds differ during electrocution, we assume that death by electrocution in broilers also supervenes rapidly, on average between 1 and 3 seconds. Evidence on precise times is not yet available. We tentatively assume that the the intensity of the pain felt during these seconds can reach an Excruciating level, as based on symptoms from victims of torture with electrical shocks and observations of the painful nature of ventricular fibrillation [79]. Moreover, different from local shock, here the current affects the entire body, as suggested from the observation of postmortem lesions throughout the entire body. Still, we do not completely rule out the possibility of Disabling pain only (Pain-Track 9)

Pain-Track 9. Hypothesis for the pain associated with fatal electrocution (cardiac arrest) in broilers. The table on the right shows the estimated time in pain as a result of this experience.

	Stun-kill	TIME IN PAIN (sec)
Excruciating	90%	1.50 [0.75-2.25]
Disabling	10%	0.50 [0.25-0.75]
Hurtful		
Annoying		
1-3 s		

III. 3 Pain associated with Neck Cutting in Conscious Birds

In poultry, neck-cutting by severing both carotid arteries and external jugular veins leads to rapid blood loss: when severing is effective, high pressure jets of blood should flow for approximately 5-10 seconds [80]. The interruption of the supply of oxygen results in progressive brain dysfunction, eventually leading to loss of consciousness and brain death.

In humans, neck-cutting by a sharp blade is deemed as one of the fastest methods to induce brain death [81,82]: severing of the carotid by slashing results in death in approximately 5-15 seconds [81,83]. In cattle, evidence indicates that it will take 10 seconds up to over 3 minutes for non-stunned animals to lose consciousness after the cut [84]. In broiler chickens that were previously stunned, the severing of both common carotid arteries and jugular veins has been shown to lead to a quiescent electroencephalogram (EEG) within approximately 15 – 30 seconds [36]. Several factors may, however, modulate the rate of blood loss and therefore these times, such as the stunning method and voltage parameters used [85]. In those cases where not all vessels are properly severed, bleeding will take longer, extending the time to the onset of unconsciousness [80]: in birds with only one carotid artery and one jugular vein severed, a quiescent EEG may take four times longer, from 1 to 2 minutes, to be achieved [36].

In this section we are concerned with the pain endured by individuals not properly stunned (still conscious).

Duration of experience

In non-stunned birds, evidence based on EEG activity and standing posture [86] provide an idea of expected times to loss of consciousness following neck cutting. In one experiment mimicking commercial conditions, observed times to loss of consciousness were 19 (11 - 27) seconds after both carotid arteries were cut [87]. In an investigation of the (kosher) slaughter of broilers, loss of posture occurred 8 to 26 seconds after neck cutting [88]. The proposed hypothesis for the onset of unconsciousness in Pain-Track 10 encompasses the range of times reported in non-stunned birds (5-30 s). In Pain-Track 11 we consider those cases of improper severing of the arteries and veins, in which case the time to the onset of loss of consciousness is assumed to be four times longer [36] (20-120 s). **Unfortunately, to our knowledge there is no data available on the proportion of non-stunned birds that have their arteries ‘improperly’ severed. We tentatively estimate that the average will lie somewhere in the interval 5-30%.** Again, data is very much needed to estimate more precisely typical proportions of birds affected by the challenges discussed here.

Intensity of experience

It is widely acknowledged that slaughtering conscious animals by neck incision is painful [86,89–91] (it has been, in fact, the recognition of this painful nature that led to legislation on the need of stunning before bleeding). Evidence that enables inferring the intensity of the pain associated with neck cutting is, however, scarce. Bleeding to death is not necessarily painful by itself. It is the injury resulting in bleeding or, in the case of internal hemorrhages, the expansion of internal cavities, that causes pain [92]. Since the onset of death is relatively fast, we have grouped in Pain-Tracks 10 and 11 both cutting and bleeding into a single phase in which the intensity of pain remains invariable while it lasts, before consciousness is lost [82].

In humans, anecdotal reports by stabbing survivors [93] suggest that cuts with sharp blades are hardly painful in the first fractions of second after the injury is inflicted. The trauma caused during neck cutting will trigger a local inflammatory reaction on the skin, muscles, vessels, trachea and peripheral areas, producing hyperalgesia at the site of incision and secondary

hyperalgesia in the area surrounding the cut [94]. Pain caused by incisions, or “incisional pain”, has been widely studied in the context of surgical procedures and is known to increase with time in the absence of analgesia [94]. Neck incision has also been proven to be painful in anesthetized calves during the 30 seconds following incision, based on EEG responses [95,96]. Therefore, we assume that incisional pain before loss of consciousness will be predominantly Disabling (70%). Still, some authors argue that clean incisions made with exquisitely sharp blades could minimize pain and distress [97]. To account for this possibility, we consider a 20% likelihood of Hurtful pain and a 10% likelihood of Annoying pain in Pain-Tracks 10 and 11.

Pain-Track 10. Hypothesis for the intensities and temporal evolution (column on the left) of the pain associated with the **proper incision of vessels and carotids in conscious birds**. The column on the right shows the estimated time in pain of each intensity as a result of this experience. Proportion of birds enduring this experience: 70-95% of non-stunned/killed birds.

	Incisional pain (severing effective)	Time in Pain (sec)
Excruciating		
Disabling	70%	12.25 [3.50-21]
Hurtful	20%	3.50 [1-6]
Annoying	10%	1.75 [0.5-3]

5 - 30 sec

Pain-Track 11. Hypothesis for the pain associated with the **improper incision** of vessels and carotids in conscious birds. The table on the right shows the estimated time in pain as a result of this experience. Proportion of birds enduring this experience: 5-30% of non-stunned/killed birds.

	Incisional pain (improper severing)	TIME IN PAIN (sec)
Excruciating		
Disabling	70%	49 [14-84]
Hurtful	20%	14 [4-24]
Annoying	10%	7 [2-13]

20 - 120 s

III.4 Pain associated with the Scalding of Conscious Birds

In poultry production, scalding is the process of submerging the birds’ bodies into boiling water to loosen and facilitate the removal of feathers [98]. ‘Hard’ scalding methods use water temperatures from 56 to 66°C and immersion times of 45 to 120s, while soft scalding methods use temperatures from 51 to 54°C and longer immersion times (120 to 220s) [28,99]. Hard scalding is typically used in the United States.

Unfortunately, failures in previous stunning stages will lead to a proportion of chickens entering the scalding tank while still alive and conscious [42,100].

Once submerged in the scalding tank, boiling water will cause direct trauma and burn the skin of the entire body. Pain is triggered immediately upon exposure given the dense innervation of the skin surface with sensory nociceptors [101]. Reports from burn patients indicate that the

degree of pain is usually proportional to the degree of tissue damage [102,103]. There is superficial epidermal burn (including eyes, mouth and respiratory tract), followed by full dermal burn and subsequent damage of internal tissues and organs. Burns affecting both epidermis and dermis are categorized as third degree burns [104]. If underlying tissues (e.g. muscles, bones) are affected, the injury is categorized as fourth degree burn. In third and fourth degree burns nerves are also damaged, so there is no feeling in the affected areas [105], but until then, the pain is widely recognized as excruciating. Burn pain is recognized as one of the most severe types of pain, comprising nociceptive, inflammatory, and neuropathic components [106,107]. Death by boiling was used as a method of execution in the Middle Ages given its extremely painful nature [108,109]. Because of the nature of the injuries and their extension, which affect 100% of the skin, we assume that conscious birds will experience pain of an Excruciating level until death or total damage of nerve endings, whichever comes first [110,111] (Pain-Track 12).

It could be hypothesized that soon after being submerged into the scalding tank the bird might go into shock, as extreme pain can trigger a neurogenic shock as a compensatory mechanism by overexcitation of the parasympathetic nervous system, to maximize blood flow to the most important organs and systems in the body [112]. However, we find this possibility unlikely, as during scalding the whole body is compromised, impairing these compensatory mechanisms. Additionally, in scalding accidents involving people and animals who fell into hot springs, bystanders have often reported that victims stayed conscious throughout the several minutes that the experience lasted, screaming, calling for help, or trying to escape [113,114]. Similar reports exist for witnesses of punishment by boiling (it is the very fact that victims were known to suffer for a long time that made this a chosen form of torture and punishment throughout human history). This strongly supports the notion that the bird will be conscious throughout the scalding experience, being able to feel pain until the fatal outcome [115].

Another hypothesis is that the bird could die by drowning first. However, we find this possibility unlikely since death by drowning tends to be relatively slow [116]. The time to death by drowning has been measured in the laboratory for minks (*Mustela vison*), muskrats (*Ondatra zibethica*), and beavers (*Castor canadensis*) [116]. EEG and electrocardiograph (ECG) readings were recorded during drowning and time of death taken as the moment when the EEG signal became flat. On average, the EEG signal became flat in minks after 4 min 37 sec; in muskrats after 4 min 3 sec; and in beavers after 9 min 11 sec. Therefore, we hypothesize that the birds that enter the scalding tank alive will die first from multiple organ failure. We hypothesize that time to the onset of unconsciousness should range from 20 to 120 seconds (from submersion of the bird into the scalding tank until death), the time typically used in hard scalding methods.

Pain-Track 12. Hypothesis for the pain associated with the scalding of conscious birds. The table on the right shows the estimated time in pain as a result of this experience.

	Scalding	TIME IN PAIN (min)
Excruciating	100%	1.17 [0.34-2]
Disabling		
Hurtful		
Annoying		
	20 - 120 s	

III.5 Pain from Fear from the moment of shackling until loss of consciousness

Fear is an adaptive response that helps individuals react appropriately when faced with threats to their physical integrity and/or reproductive capability. Despite its adaptive nature, fear can be a powerful and damaging stressor if intense, or if the animal is prevented from properly responding to the threat [117]. Indeed, fear is widely regarded as a state of suffering [117].

Intense fear during the highly stressful process of stunning and slaughter is expected. As discussed previously [10], in the Cumulative Pain framework assessment of pain experienced by an individual over a period of time (in this case, during stunning) is represented by the sum of the time spent in pain due to all welfare challenges experienced, both sequentially and concurrently. Here, we assume that fear is experienced concurrently with physical pain, being predominantly of a Disabling nature, as it is expected to capture all the bird's attention given and inhibit other motivational systems (Pain-Track 13). The only exception is in the case of scalding, in which case we tentatively assume fear is equally likely to be Disabling or Excruciating given the nature of the experience as discussed and justified in section III.4.

Pain-Track 13. Hypothesis for the pain associated with **fear**. The proportion of birds enduring this experience is shown in Table 4.

	Shackling	Waterbath	Stun to neck	Bleeding	Scalding
Excruciating					50%
Disabling	75%	75%	75%	75%	50%
Hurtful	25%	25%	25%	25%	
Annoying					
Duration	20-90s	1-2 (effective) 4-24 (ineffective)	3-18	5-30 (proper) 20-120 (improper)	20-120

The implications of fear, however, may go beyond its aversive nature. Fear-induced analgesia is known in many species [118–120], with evidence that it may also be present in chickens. In Box 2 we discuss this possibility. Should it hold, it would have major implications for the understanding of pain in events associated with intense fear, such as the slaughter process. The need and implications of further research in this area cannot be stressed enough.

Box 2. Tonic immobility triggered by fear: potential effects on pain and implications for the understanding of pain during slaughter

Chickens are known to react to a number of fear- and pain-eliciting stimuli by going into a state of complete motor paralysis, known as 'tonic immobility' (TI), an involuntary reflexive reaction characterized by a reversible, but profound, motor inhibition, the duration of which is positively related to the level of fear of the birds [19].

From an evolutionary perspective, tonic immobility is believed to have evolved as a response to predator attacks after flight and fight responses have failed - a last resort in face of inescapable danger. Accordingly, some studies [121,122] have shown that tonic immobility can increase prey

survival by reducing the time of handling by the predator (during which escape is possible) or by increasing the likelihood that predators will drop or cache the individuals for consumption at a later time, providing them with further opportunities to flee. Tonic immobility is observed in a wide variety of vertebrate and invertebrate species, from ants and spiders to fish, rodents and even humans, such as victims of sexual assaults [123]. In chickens, tonic immobility has been shown to be elicited by both fear-inducing and painful stimuli [124].

There is some evidence that chickens may experience reduced pain while in this paralyzing state [125]. In one study [126], EEG measurements showed a characteristic high-amplitude low-frequency (HALF) activity during this period of immobility similar to that seen in sleep and in catatonic states like tonic immobility. A similar pattern has been reported for general anesthesia in humans, where a progressive increase in HALF activity is observed as the level of anesthesia deepens [127]. These observations would suggest that chickens may experience reduced or no pain when in this state [125]. In general, the altered electroencephalogram patterns persist for short intervals after the triggering event, alternating with periods of normality [126]. This is likely an adaptive response to ensure the animal has the possibility of regaining awareness and escape from an attacker, but it may also mean that analgesia may be frequently interrupted during an attack.

Analgesia in situations of tonic immobility has been reported for other species [128,129]. In guinea pigs, for example, tonic immobility has been shown to induce endogenous analgesia involving opioid synapses, which increased their threshold to noxious stimuli such as heat [119]. In fact, analgesia induced by stress and situations of severe pain has been documented in many species (including humans [130]), in situations of tonic immobility and others, such as uncontrollable footshock, restraint, inescapable swimming in cold water and body pinching [131], presumably as a mechanism protecting the organism from being overwhelmed by pain during a dangerous situation.

Still, conclusive evidence as to whether tonic immobility has analgesic effects in chickens is still lacking. Additionally, it is not possible to completely rule out the possibility that immobility emerges as a result of tonic immobility or learned helplessness [125], a phenomenon in which an animal exposed repeatedly to an inescapable stressor gives up reacting, with no attempts of withdraw or protection, even when escape becomes possible.

Further studies are critically needed to determine the extent to which pain is suppressed in situations of extreme fear. Should this possibility hold, it would have major implications for the understanding of pain and suffering, particularly during the process of stunning and slaughter.

IV. Controlled Atmosphere Systems (CAS)

CAS methods eliminate the need to manually handle live birds and are less sensitive to variations in bird size and body conformation, which affect stunning effectiveness. By eliminating the handling of birds, CAS methods also prevent welfare issues related to poor stockmanship and animal abuse [33]. Still, some of the gases used for stunning poultry are aversive, leading to respiratory distress, anxiety, fear, and pain [132], since loss of consciousness is not immediate, but progressive.

Upon arrival in the slaughterhouse, the handling of birds and their transport to the stunning points will largely depend on the features of the system used. In most cases, birds arrive at the slaughterhouse in modules or single drawer systems designed to be placed directly in chambers that are either pre-filled or subsequently filled with gas [33,133,134]. In older systems, birds are removed from the containers onto a conveyor belt, which moves them

loosely through the stunner [33]. Birds can be also introduced into the chamber through openings in the top [135]. Once the stunning process is complete, birds are removed from the transport crates, hung on the shackles, bled and scalded in the same way as birds exposed to electrical waterbath stunning.

There is a great variety of gas stunning systems available which differ on the design, number of phases and gas mixtures used. Designs are primarily of three types: tunnels and pits (which are pre-filled with gas) and closed cabinets, where gas is introduced once the animals are inside. In tunnel systems, birds are transported through a tunnel, where they are exposed to increasing gas concentrations on a varying number of phases, or 'stations' [33,136]. The final station contains a higher concentration to ensure death [33]. In pit systems, birds in drawers are placed inside a pit where the gas is injected at the base. Gravity holds the (heavier than air) gas at the bottom of the pit [33]. Birds are moved downwards deeper into the pit by means of a lift system, so they are exposed gradually to increasing gas concentrations [137]. Finally, close cabinets allow the birds to remain inside containers, eliminating all handling. Close cabinets also allow for a more precise calibration of gas concentrations [136].

Gas mixtures used for stunning poultry are: carbon dioxide (CO₂) delivered in gradually increasing concentrations, in two or more phases; inert gases (nitrogen, argon or both, with a maximum of 2% residual oxygen); and mixtures of carbon dioxide (up to 40%) with inert gases and or oxygen [11]. Low atmospheric pressure stunning (LAPS), approved for use in the European Union in 2018 [138], is another stunning method in which birds are exposed to gradual decompression, similar to the operating principle of a vacuum pump, reducing oxygen levels to less than 5%. The stunning or killing of the birds is the result of the lack of oxygen, which causes loss of consciousness and, depending on the parameters used, the subsequent death of the birds.

In systems with gas mixtures, when gas concentrations are high and exposure is long enough, the birds will be killed³ [33] (sometimes referred to as controlled atmosphere killing, CAK). With CAS methods the stun-kill interval (Table 1) is longer than with electrical stunning. Parameters such as gas concentration, particularly when carbon dioxide (CO₂) is used, the number and duration of phases, and gas temperature will all affect the time to the onset of unconsciousness and the welfare of the birds until then. If CAS stunning is effective, the relaxed birds' bodies are shackled, their neck cut and they are bled to death.

Table 1. Typical duration of the stunning procedure for different Controlled Atmosphere Systems (CAS) methods, gas mixtures and number of phases (with increasing gas concentrations).

Design	Method	Duration (min)	Source
Tunnel	CO ₂ : 2 phases	2	[25,139]
	CO ₂ : 3 phases	3-3.5	[32]
	CO ₂ : 5 phases	5-7	[32]
	CO ₂ and inert gas	1-3	[11,140]
	Inert gas	1-3	[11,140]
Pits		5.5-6.5	[137]
Close cabinets		6	[141]

³ The killing of birds is often referred to by terms such as 'irreversible stunning', or by mentioning that birds 'never resume breathing'.

In this section, we examine the welfare issues associated with CAS methods, focusing on those based on the use of carbon dioxide, the most commonly applied stunning gas under commercial conditions [134,142].

IV. 1 Gas stunning with CO₂

In commercial poultry production, the most widely used stunning gas is carbon dioxide (CO₂), an anaesthetic gas that induces unconsciousness [134,142]. CO₂ is a by-product of the manufacture of ammonia fertilizers and ethanol fuel [143], is relatively cheap, denser than ambient air (hence easy to contain in unsealed chambers) and also the method of choice for the killing of laboratory animals.

When CO₂ is inhaled, it dissolves in the water in the lungs and enters the bloodstream. When combined with water, CO₂ forms carbonic acid, which causes respiratory acidosis, and rapid acidification of the cerebrospinal fluid and the brain cells, depressing brain activity. Loss of consciousness is not immediate. Prior to that, CO₂ activates pathways in the brain that elicit anxiety, fear, wing flapping, headshaking, and gasping for air [33,132,144,145] (Table 2). The higher the concentration, the higher the number of aversive behavioral responses observed [146,147]. In humans, symptoms associated with respiratory acidosis include headache, confusion, anxiety, and drowsiness, followed by dyspnea, vomiting, disorientation, hypertension, and loss of consciousness [148]. Experiments with rats have shown that CO₂ concentrations in the range of 10-15% already induce acute toxicity and may be fatal [149]. Broilers show headshaking and respiratory distress already at a concentration of 10% [146]⁴, followed by the eventual 'loss of posture', to which birds may react by wing flapping to regain balance. Further gasping and convulsions have also been observed after this stage [11]. Table 2 shows standard behavioral reactions ranked by welfare experts in the poultry industry.

Table 2. Welfare ranking of behavioral reactions during stunning [32,33,150].

Behavior	Ranking
No change in behaviour from the time birds enter the stunner until they lose posture.	Excellent
Gasping only, with no other change in behavior from the time the birds enter the gas until they fall over (lose posture); Most birds with no wing flapping and a few birds with weak intermittent flapping.	Acceptable
Gasping, combined with a continuous wing flapping from the time the birds enter the gas until they fall over (lose posture)	Not acceptable
All birds flap continuously or attempt to climb out of the container from the time the birds enter the gas until they fall over (lose posture)	Serious problem

The greatest challenge with the use of CO₂ stunning is finding a good balance between the time to the onset of unconsciousness and the amount of distress for the birds [151]; while the faster provisioning of high concentrations of CO₂ will lead to loss of consciousness more swiftly, it can be highly aversive to the birds [33].

Therefore, the use of multiphase stunning models, which start with a lower CO₂ concentration that is progressively increased, has been widely acknowledged as an alternative to minimize the distressing effects of sudden exposure to high concentrations of CO₂ [32]. In Europe, for example, it is compulsory to expose birds to CO₂ gradually, in at least two phases of increasing

⁴ Carbon dioxide is normally present at a 0.04% concentration in normal room air.

concentration: a first phase with up to 40% CO₂, where loss of consciousness is induced through narcosis, followed by a second phase where the concentration of CO₂ is increased up to 80%–90%, inducing a deeper state of unconsciousness [6,134,151].

Still, although allowed and used in commercial operations, biphasic CO₂ stunning is not recommended by some authors. Instead, a greater number of phases is often advised to minimize distress [33]. New generation systems with a five-stage cycle of 5-6 minutes have been shown to be less aversive than cycles of fewer phases [32,136], though aversive responses are still observed until unconsciousness [151].

The effectiveness of stunning with CAS is not, however, always ensured. As with other systems, some birds might not be rendered unconscious if gas concentrations are not properly adjusted, or exposure time is insufficient. For instance, if production is expanded and the gas stunner has a small capacity, there is a risk that operators speed up the process, reducing exposure time [152]. In fact, having an undersized machine has been considered the number one hazard when it comes to CAS methods [33].

In the next sections we investigate the welfare impact of the more modern five-stage systems, adopted in commercial operations. In these systems, a gradual increase of CO₂ is achieved over a period of 5-6 minutes by exposing birds to an initial concentration of approximately 20 vol% CO₂ for about one minute, which is then increased by about 10% in each of the 1-min stages.

IV. 1. 1 Pain in Properly Implemented Multistage Carbon Dioxide Systems

To our knowledge, there are only two publications that report on the behavioral responses of broilers exposed to multistage systems in commercial conditions, in which the results are disaggregated for each of the stunning stages [151,153]. We use these studies to determine the typical times of exposure to each CO₂ concentration, as well as the behavioral responses observed in each stage. Their observations are described in Table 3.

Table 3. Onset (in seconds) of behavioural indicators of aversiveness and unconsciousness in broilers exposed to five-stage stunning systems (similar to those commercialized by Meyn and Marel) with carbon dioxide indicators, as measured in [151] (a) and [153] (b). *Experimental conditions.

Indicator	Stages and corresponding CO ₂ concentrations				
	1: 20% CO ₂	2: 30% CO ₂	3: 40% CO ₂	4: 50% CO ₂	5: ≥60% CO ₂
Alertness	11-17 s				
Head flicks	11-17 s				
Sneezing	11-17 s				
Gasping	13 s				
Head Shaking	18 ^a , 16 ^b s				
Deep Breathing	16 ^b s				
Wing Flapping		66 s			
Loss of Posture		66-93 ^a , 76 ^b s			
Convulsions		93 s			
Loss of α and β waves*		60 ± 23 s			
Duration (s)	60 s	60 s	60 s	60 s	120 s
Timeline (s)	0-59	60-119 s	120-179 s	180-239 s	240-300 s

The aversive effects of CO₂ are of different types. The first are those caused by the production of carbonic acid when CO₂ is dissolved in water, and the resulting irritation of the respiratory and mucous membranes of the nose and throat [148,154,155]. Carbonic acid activates nociceptors in mucous membranes and free endings of nerves, which are especially sensitive to painful stimuli as they are not covered by squamous epithelium [156–158]. Additionally, CO₂ also leads to stimulation of intrapulmonary chemoreceptors which increase respiratory frequency, triggering symptoms of suffocation and ‘air hunger’, a conscious feeling of severe breathlessness in response to failure to maintain sufficient oxygen supply [159].

In the observations described in Table 3, gasping (intermittent or forceful inspiratory movements [160] associated with breathlessness and reduced welfare [155,161]) was observed as early as 13 seconds, with deep breathing starting at 16 seconds. Head shaking (rapid side-to-side movement of the head) started soon after. Head shaking is typically considered an indicator of the aversive nature of CO₂ [144,151,162,163], as treatments with air do not induce this behavior [146,164] and the effect of CO₂ on headshaking is dose-dependent [146,162]. Head shaking is also often observed soon after exposure to substances with known irritant properties [165], although some authors have linked this behavior with a state of alertness [166,167].

The observation of gasping soon after CO₂ exposure suggests that the discomfort (pain) experienced under 20% CO₂ is at least of a Hurtful nature (Box 1), as the sensation of breathlessness cannot be easily ignored. A rapid onset of gasping at low CO₂ concentrations has been also observed by other authors [144]. From an evolutionary perspective, the degree of unpleasantness associated with impaired air intake should be high enough to ensure that less critical ongoing behaviors and processes are put on hold, as failure to attend to this need is fatal.

The hypothesis of at least Hurtful pain with 20% CO₂ is further supported by observations that chronically feed-deprived broiler breeders exposed to CO₂ rapidly stopped eating (12 seconds after exposure), at a point when CO₂ concentration was only 3% [144]⁵. As we discuss in Chapter 6, chronic feed restriction in broiler breeders is associated with multiple neurophysiological and behavioural indicators of stress [169]. The observation that hungry feed-restricted broiler breeders stop feeding soon after exposure to CO₂, but not to other gases (e.g., N₂, medical air) thus supports the expectation of a high intensity of pain. Accordingly, we set a 80% probability of at least Hurtful pain, split equally into Hurtful and Disabling pain. Still, behavioral observations show individual variation in responses, potentially due to individual differences in susceptibility to CO₂'s effects. To account for this variation, we also consider a lower likelihood of Annoying pain in this first stage of exposure. The duration of the aversive experience is set at 40-50 seconds, since discomfort only starts 10-20 seconds after the delivery of the gas [151,153].

When CO₂ concentration reaches 30% (second stage), different scenarios are possible: (1) the sensation of breathlessness may be intensified (hence the greater probability of Disabling pain in Pain-Track 14). The greater likelihood of Disabling pain is reinforced by the observation of wing flapping in some birds, a behaviour interpreted as an indication of severe distress and pain by some authors [170,171]. Alternatively, for some birds, the progressive central nervous system depression from CO₂ exposure may ‘begin’ to reduce sensory perception of

⁵ Different from the experiments cited, other choice experiments tested the extent to which broilers remained in a hypercapnic environment where feed was provided. However, active evacuation of the test places in such cases required the birds to move out of the test chambers, which may have been prevented by impairment of the birds' locomotor and cognitive ability caused by the sedative and narcotic effect of CO₂ [168].

breathlessness in this stage, though this effect is likely to develop only after an initial period of intense respiratory distress, hence a low (20%) probability attributed to the Annoying category. Such a lower intensity is also expected if the progression from full consciousness to unconsciousness is not completely binary, with intermediate brain states. The fact that acidosis progressively affects neural function further suggests there are likely gradations of consciousness impairment before complete unconsciousness. To account for the fact that many birds will have lost consciousness before the end of the second stage (as soon as 6 seconds after the increase of CO₂ concentration to 30%, Table 3), we also attribute a 30% probability of no pain in stage 2, which represents unconscious birds.

Loss of posture (LOP, Table 3), occurred at 6 to 33 seconds in this second stage. Evidence indicates that birds are conscious for 20-40 seconds after LOP [172,173] (Box 4). This means that birds may be likely conscious (using the data from Table 3) for a minimum of 26 seconds after initiation of the second stage, and a maximum of 73 seconds (60 seconds in the second stage, and possibly up to 13 seconds in the third stage, when CO₂ rose up to 40%).

Although α and β waves were suppressed in all birds before the third stunning stage (40% CO₂), interpretation of EEG patterns is challenging (Box 4), so we cannot completely rule out the possibility that a few birds were still partially conscious in the beginning of the third stage. Still, we limit that percentage to 5% (hence attribute 95% probability to 'no pain' in stage 3 of Pain-Track 14). In the third stage, two possibilities are envisioned: (1) pain is of a Disabling nature for the few birds that will experience given the intense state of breathlessness at this CO₂ concentration; (2) pain is less intense (Hurtful), given the progressive state of numbness/sedation caused by the gas. Both possibilities are considered to be equally likely. We assume that under properly implemented commercial (CAK) conditions as many as 99.7% of all birds will have died in this third stage [155], hence no further stages are considered.

Pain-Track 14 summarized the hypothesis for the evolution and intensity of the pain associated with each of the stunning stages.

Pain-Track 14. Hypothesis for the pain associated with exposure to a five-stage CO₂ stunning system. Pain in atmospheres where CO₂ is at a 50% concentration or higher is not estimated as birds are expected to be unconscious. The table on the right shows the estimated time in pain as a result of the experience.

	1: 20% CO ₂	2: 30% CO ₂	3: 40% CO ₂	4: 50% CO ₂	5: >60% CO ₂		TIME IN PAIN (sec)
Excruciating						Excruciating	
Disabling	40%	60%	2.5%			Disabling	8.81 [5.41-12.21]
Hurtful	40%	20%	2.5%			Hurtful	42 [35.74-48.26]
Annoying	20%					Annoying	22.8 [18.8-26.8]
Unconscious		20%	95%				
	40-50 s	26-60 s	1-13 s	--	--		

Box 3 Indicators of unconsciousness used in CAS research

Loss of posture (LOP) has been traditionally considered the main behavioral indicator of loss of consciousness. LOP is certainly correlated with loss of consciousness, as it precedes it, but does not equate unconsciousness. Different LOP's definitions are in use, including "the inability to maintain a sitting position and neck tension" [172], "the cessation of standing with the head resting against either in the floor or wall" [144] or the "inability to regain/maintain a controlled posture" [174,175]. None of these definitions involves cessation of movement. Additionally, objective scoring of LOP has proven challenging, as the birds' movements are difficult to observe and it is often not possible to distinguish between sitting and LOP [151,172]. Importantly, although some authors equate LOP to loss of consciousness, EEG measurements indicate birds may still be conscious for 20-40 seconds after LOP [172,173].

Electroencephalogram patterns: EEG monitoring is regarded as the most reliable method for assessing consciousness [176,177]. There are four wave patterns in EEGs [178]: alpha (α), typically observed in conscious individuals; beta (β), associated with alertness, and the slow waves delta (δ) and theta (θ), which indicate reduced consciousness (often present during sedation and sleep [172]). Suppression of alpha and beta waves with occurrence of delta and theta waves is interpreted as an indicator of unconsciousness [151], whereas an iso-electric (flat) EEG indicates brain death [178]. Interpretation of EEG patterns is, however, challenging. In humans, EEG monitoring for measuring anesthetic depth is still difficult despite the availability of sophisticated equipment [177]. In animals, EEG patterns are easily disturbed by technical artefacts (e.g. placement of electrodes [179]) and animal-related issues (e.g., eye or muscle movements) [180]. Interpretation is also heterogeneous, with some authors relying only on wave patterns in raw EEGs [181], whereas others favor processed measures, such as the correlation dimension [173] or spectral analysis [172,175,182]. Determining the onset of unconsciousness in CAS based solely on EEG activity is even more challenging, since changes in wave patterns are gradual.

Electrocardiogram patterns: ECG recordings have also been used to assess behavioral arousal, consciousness and death in CAS research. In particular, absence of increased heart rate in hypobaric hypoxic stunning (LAPS) has been interpreted as an indication of lack of stress [175]. It is well established, however, that hypoxia prompts complex compensatory cardiac responses depending on multiple physiological parameters [183]. While internal chemoreceptors produce bradycardia and peripheral vasoconstriction, both characteristic responses to hypoxia, these responses might be modified by ventilation and other physiological factors [184]. It is therefore risky to infer absence of stress or pain based on no observed tachycardia in an hypoxic environment.

The Effect of Fear

In mammals (including humans), hypercapnic environments with as little as 7% CO₂ are associated with sensations of fear, anxiety, and panic [185,186]. In rats, brain regions and neurotransmitters involved in negative emotional responses are activated by hypercapnia and acidosis associated with CO₂ exposure [186]. Responses to CO₂ are also counteracted by the administration of anxiolytics [186]. Fear and anxiety are therefore additional negative effects of CO₂ if these findings can be generalized to poultry. Whether the neural circuitry for fear in birds is activated by exposure to hypercapnic gases has not yet been investigated. However, since in mammals fear is triggered through the detection of carbon dioxide and acidosis by the amygdala, an extremely ancient structure also present in birds, a similar mechanism is most likely to be present in chickens [187].

Therefore, we consider the possibility that birds experience fear while exposed to CO₂ (Pain-Track 15). We assume that the intensity of fear is lower (Hurtful) than that assumed for birds shackled and stunned in electrical waterbath systems (Disabling, see Pain-Track 13). Moreover, estimated probabilities are modulated downwards to account for the progressive loss of consciousness aimed with this stunning method. The estimated duration of the experience is the same as that estimated for the duration of the aversiveness caused by CO₂ in Pain-Track 14.

Pain-Track 15. Hypothesis for the fear associated with exposure to a five-stage CO₂ stunning system. Pain in atmospheres where CO₂ is at a 50% concentration or higher is not estimated as birds are expected to be unconscious. The table on the right shows the estimated time in pain as a result of the experience.

	1: 20% CO ₂	2: 30% CO ₂	3: 40% CO ₂	50% CO ₂	>60% CO ₂		TIME IN PAIN (sec)
Excruciating						Excruciating	
Disabling						Disabling	
Hurtful	50%	30%				Hurtful	37 [31.7-43.3]
Annoying	50%	30%	5%			Annoying	36.6 [30.3-43]
Unconscious		40%	95%				
	40-55 s	26-60 s	1-13 s	--	--		

Stunning Effectiveness in Properly Implemented Systems

When properly implemented, multistage CAS stunning renders most of the birds unconscious. In observations of the five-stage system of Meyn under commercial conditions [153], effectiveness, defined as the proportion of birds which were rendered unconscious during the procedure, was 99.97%. In another system tested in Germany, in which broilers in their transport crates were exposed to slowly increasing concentrations of CO₂ in air up to between 50 and 65% (with total times between 4.5 and 7 min), stunning efficiency was 99.98% [188]. The authors also observed those cases of failure of automatic cutting and the percentage of birds awake before scalding. In this system, 0.017% of birds were awake before scalding [188].

Therefore, to quantify the pain endured by those animals that regain consciousness after the stunning process, we assume that 0.02 to 0.04% regain consciousness after leaving the stunner. In such cases, the pain from neck cutting and bleeding is as described in Pain-Tracks 10 and 11. Similarly, we assume a 0.01 to 0.02% probability of birds entering the scalding tank still conscious. Pain in this case is as described in Pain-Track 12.

IV. 1. 2 Pain in poorly managed systems

As discussed previously, ineffective stunning with CAS is likely to occur if gas concentrations are not properly adjusted, or exposure time is insufficient. An interval between two and three minutes is necessary to ensure that birds are “*irreversibly stunned*” (killed) in CAS systems with CO₂ and other gas mixtures. This interval is considerably longer than that needed in electrical waterbath systems. This has led to concerns on whether slaughter plants will be willing to leave

birds in gas mixtures for such a long time, given the typical prioritization of speed and high throughput rates [73,189]. The risk of a short exposure is heightened if production is expanded and the gas stunner has a small capacity, in which case, operators may speed up the process [33,152].

Short exposure is likely to result in large numbers of birds regaining consciousness before neck cutting, particularly considering the relatively long time that it takes to shackle the large number of birds leaving the stun unit. That is, the duration of unconsciousness is likely to be shorter than the time interval between the end of stun and the end of the bleeding stage. Other factors may also affect the effectiveness of stunning, such as a reduced concentration of CO₂ in the final stages and even lairage times [188].

Here we also simulate the effect of poorly managed CO₂ systems on broiler welfare during stunning. To this end, we assume that the aversive experience associated with exposure to CO₂ inside the stunning unit is the same as that described in Pain-Track 14, but that a fraction of birds regain consciousness owing to the exposure time being insufficient to ensure that birds are killed before leaving the stunner.

To our knowledge, there have been no measurements of the proportion of birds regaining consciousness depending on the time of exposure to CO₂ in commercial multistage systems. In the absence of data, we simulate the additional time in pain experienced for a wide range of values, assuming that 1% to 30% birds may regain consciousness in poorly managed systems. In such cases, these birds will experience pain from neck bleeding (as determined in Pain-Tracks 10 and 11). We also simulate the additional time in pain if a fraction of these conscious birds (from 0.1-6%, the same percentage as that estimated for birds improperly stunned in electrical waterbath systems) experience pain from scalding (as determined in Pain-Track 12). Fear in conscious birds is assumed during bleeding and scalding, as determined in Pain-Track 13. The welfare impact of poorly managed CO₂ systems is described in the next section.

Additional welfare challenges may also emerge from the need to identify birds dead on arrival (DOA) at the slaughter plant before the birds are stunned. If crates are opened to identify and remove dead birds, the potential for additional fear, injuries and animal abuse will be present. Here we assume that no crates are opened before birds are placed in the stunning unit, even in poorly managed systems.

V. Burden of Pain in Electrical and Multistage CO₂ systems

In this section we quantify the welfare impact of electrical waterbath stunning and multi-stage CO₂ systems, as based on Pain-Tracks 1 to 15 and the assumptions described, respectively, in sections III and IV.

In the case of electrical waterbath systems, we considered three possible scenarios: (1) stunning parameters are such so that most birds are stun-killed (as employed in some European countries), (2) low voltage and high frequency stunning parameters are used to reduce the risk of carcass damage, increasing the likelihood of electro-immobilization (as often employed in the United States and other countries lacking regulation on stunning parameters), and (3) stunning parameters are appropriately set to achieve electronarcosis (effective stunning) in as many birds as possible (up to 96% effectiveness; [13]). Table 4 summarizes the assumptions for each of the three scenarios, as justified in section III.

In the case of multi-stage CO₂ systems, we considered two possibilities: (1) that the system was properly implemented, with stunning times and parameters adopted as described by the manufacturers and in the scientific literature (section IV.1.1), and (2) that the system was poorly implemented, with exposure times being insufficient to prevent birds from regaining consciousness after leaving the stunner (section IV.1.2). Table 5 summarizes the assumptions for these scenarios.

Table 4. Proportion of birds affected by aversive experiences (pain) in each phase of the electrical waterbath stunning process in the following scenarios: (1) parameters set to ensure the proper stunning of as many birds as possible (up to 96% effectiveness); (2) stunning parameters leading to the death (stun-kill) by electrocution (cardiac arrest), and (3) stunning parameters set to reduce the risk of carcass damage (low voltage, high frequency), as typically used in countries lacking regulation on stunning parameters. The proportion of individuals undergoing pain from the bleeding incision is determined as the product of conscious birds and the likelihood that the incision properly (or improperly) severs the bird's vessels. *:consciousness regained, or not stunned at all; ** parameters leading to electro-immobilization.

		Shackling	Waterbath		Bleeding Incision		Scalding
			Proper	Improper	Proper (70-95%)	Improper (5-30%)	
1. Effective stunning parameters	Proportion of birds	100%	Electronarcosis: 90-96%	Conscious*: 4-10%	3.5-8.3%	0.4-2.4%	0.01-0.6%
	Pain-Track	1-5,13	6,8,13	6,8,13	10,13	11,13	12,13
2. Stun-kill parameters	Proportion of birds	100%	Cardiac Arrest: 90-99%	Conscious*: 1-10%	1.2-8.1%	0.2-2.2%	0.01-0.6%
	Pain-Track	1-5,13	6,9,13	6,9,13	10,13	11,13	12,13
3. Parameters to reduce carcass damage***	Proportion of birds	100%	Electronarcosis: 30-95%	Conscious** 5-70%	6.9-57%	1.3-16%	0.1- 6%
	Pain-Track	1-5,13	6,8,13	6,7,13	10,13	11,13	12,13

Table 5. Proportion of birds affected by aversive experiences (pain) in each phase of the stunning process in multistage CO₂ systems. The proportion of individuals undergoing pain from the bleeding incision is determined as the product of conscious birds and the likelihood that the incision properly (or improperly) severs the bird's vessels, as discussed previously. *: consciousness regained.

		Stunner		Bleeding Incision		Scalding
		Effective	Ineffective	Proper (70-95%)	Improper (5-30%)	
Properly implemented	Proportion of birds	99.97-99.98%	0.02-0.04%	0.017-0.034%	0.0018-0.0096%	0.01-0.02%
	Pain-Track	14,15	14,15	10,13	11,13	12,13
Poorly implemented	Proportion of birds	70-99%	1-30%	1.9-24%	0.36-7%	0.1-6%
	Pain-Track	14,15	14,15	10,13	11,13	12,13

In each scenario, we determine the expected time in pain, of each intensity, endured by the ‘average bird’, namely the time in pain endured by a flock member when the prevalence of the problem in the population is considered (when 100% of a population/flock experience the pain of one welfare challenge, the time in pain endured by the average bird of that flock as a result of the challenge is the same as the time in pain endured by affected individuals; see Box 4).

Box 4 Time in Pain endured by the Average Population Member

Although pain inherently concerns individuals, we operationally accept that the collective welfare of the members of a population can also be determined. Measuring cumulative pain at the population level is also necessary to account for the heterogeneity in the exposure of population members to different challenges. For example, while the pain from shackling is experienced by all broiler chickens in electrical waterbath stunning systems, the experience of being scalded alive is undergone by a smaller fraction of individuals.

Therefore, we must consider the prevalence of each welfare challenge, so that pain (for example, from scalding) is determined for the average member of the population (which may not necessarily correspond to any real organism). At the population level, the time spent at each level of pain intensity as a result of each challenge is determined by multiplying it by its prevalence. For example, if an event causes 30 seconds of Excruciating pain in affected individuals and 10% of the population is affected, then the average member of this population could be said to experience 3 seconds of Excruciating pain due to the event. Measurements at the population level enable comparing the impact of different events and conditions across demographics, geographies and time.

Figure 4 shows the estimated time in pain endured by the average broiler chicken in the four categories of pain intensity as a result of the electrical waterbath stunning procedure and multistage CO₂ systems. In Figure 5 the same data is disaggregated for the different phases of the stunning process, and in Figure 6 the relative contribution of each stunning phase to the overall burden of pain in each scenario is shown.

In comparing electrical waterbath systems, **the use of parameters aimed at reducing carcass damage (typically low voltage and high frequency settings) translates into substantially longer times in intense pain (Excruciating and Disabling pain) as compared to waterbath systems where birds are either fatally electrocuted (stun-kill systems) or effectively stunned by electronarcosis (Fig.4).** For example, **the time endured in Excruciating pain by the average broiler in such systems is approximately three times as long as that endured in this state in the other electrical waterbath scenarios as a higher proportion of birds reaches the neck cutting blade and scalding tank still conscious (section III.2).** Since regulation of stunning parameters is lacking in most countries, including top chicken producing nations such as the United States, China and Brazil, we expect that the use of low voltage - high frequency parameters should prevail in most slaughterhouses to reduce the likelihood of carcass damage and losses.

Figure 4 also shows that **the time in Disabling, Hurtful and Annoying pain in stun-kill systems is very similar to that expected should electrical waterbath stunning be ideally**

implemented (hypothetical scenario denoted E:proper). The main difference between these systems is in the time in Excruciating pain, as the pain from cardiac arrest is not present in the ideal (hypothetical) scenario, and the values assumed for the effectiveness of stunning are higher (96-99% in E:proper vs 90-99% in stun-kill systems).

Still, the expected time endured in intense pain (Excruciating and Disabling) in properly implemented CO₂ systems is substantially shorter than in any of the electrical waterbath scenarios simulated, even an ideal waterbath system. Overall, the average broiler is expected to spend 0.02 (0.01-0.03) seconds in Excruciating pain and 8.82 (5.4-12.2) seconds in Disabling pain in multi-stage CO₂ systems, an estimate about 60 and 8 times shorter than the times in pain in ideally implemented electrical systems. Naturally, because of the relatively long time of exposure to CO₂, which is aversive to birds, the time experienced in milder states of pain (Annoying and Hurtful) is longer than in electrical systems (Figure 4).

The welfare benefits of multi-stage CO₂ stunning systems are, however, lost if exposure times or gas concentrations are insufficient to ensure that birds do not regain consciousness after leaving the stunning unit (CO₂:poor in Fig.4). In such cases, a relatively high proportion of birds may still be conscious during the bleeding stage, with some birds also experiencing the scalding process.

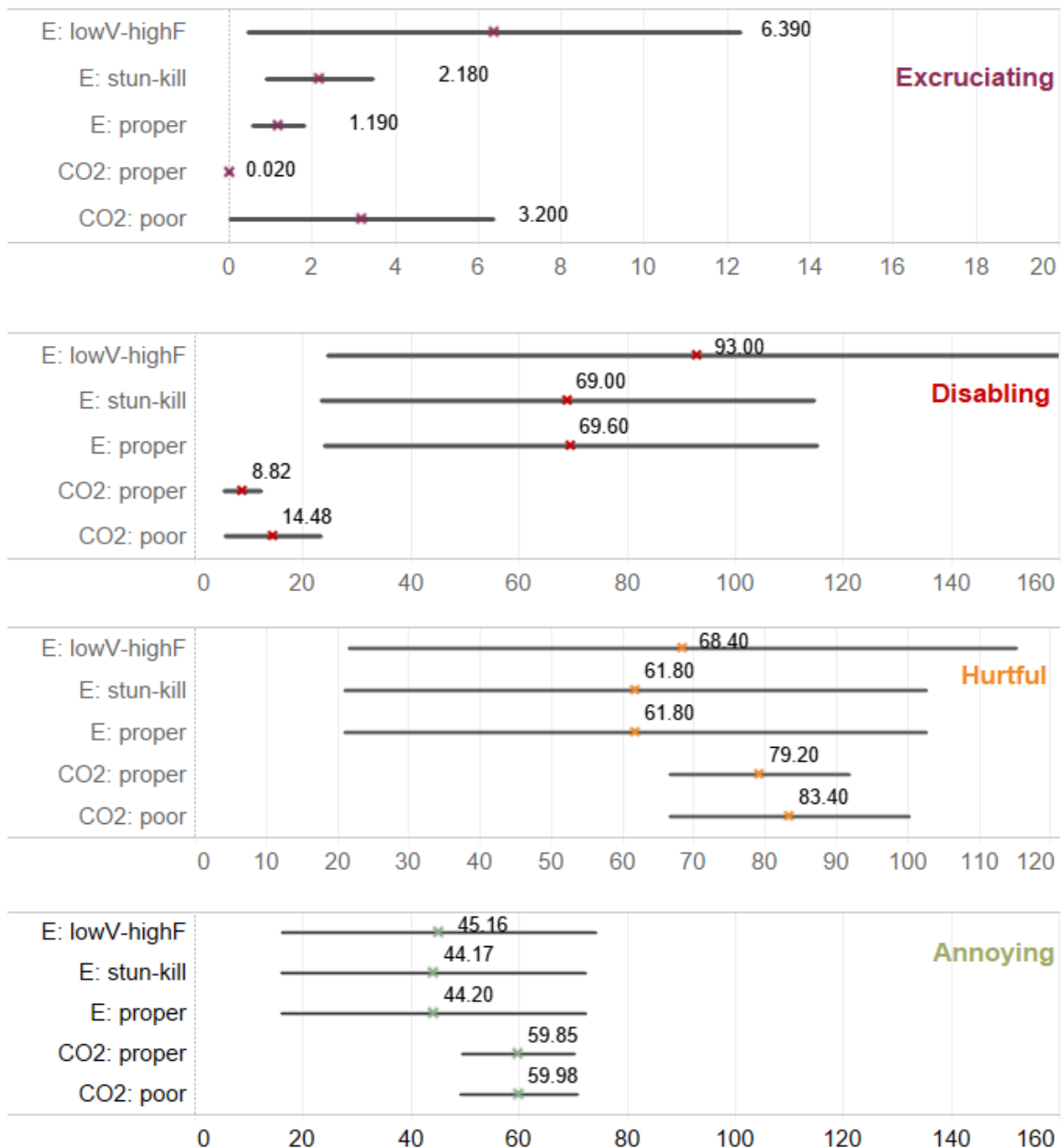


Figure 4. Expected time (seconds) in pain endured by the average broiler chicken exposed to different stunning methods: (E: lowV-highF) low voltage and high frequency stunning parameters are used to reduce the risk of carcass damage, increasing the likelihood of electro-immobilization, (E: stun-kill) stunning parameters are such so that most birds are stun-killed, (E: proper) stunning parameters are appropriately set to achieve effective stunning in as many birds as possible (CO2: proper): multi-stage CO2 system with stunning times and parameters adopted as described by the manufacturers and in the scientific literature, and (CO2: improper): exposure time (or gas concentration) being insufficient to prevent birds from regaining consciousness after leaving the stunner. Time scales are different for each category of pain intensity (defined in Box 1) for greater clarity. Error bars represent 90% uncertainty intervals. An interactive version of the figure is available at <https://pain-track.org/broilers/stunning>.



Figure 5. Expected time in pain (seconds) endured by the average broiler chicken in each phase of the stunning process in the following scenarios: (E: lowV-highF) low voltage and high frequency stunning parameters are used to reduce the risk of carcass damage, increasing the likelihood of electro-immobilization, (E: stun-kill) stunning parameters are such so that most birds are stun-killed, (E: proper) stunning parameters are appropriately set to achieve effective stunning in as many birds as possible (CO2: proper): multi-stage CO2 system with stunning times and parameters adopted as described by the manufacturers and in the scientific literature, and (CO2: improper): exposure time (or gas concentration) being insufficient to prevent birds from regaining consciousness after leaving the stunner. An interactive version of the figure is available at <https://pain-track.org/broilers/stunning>.

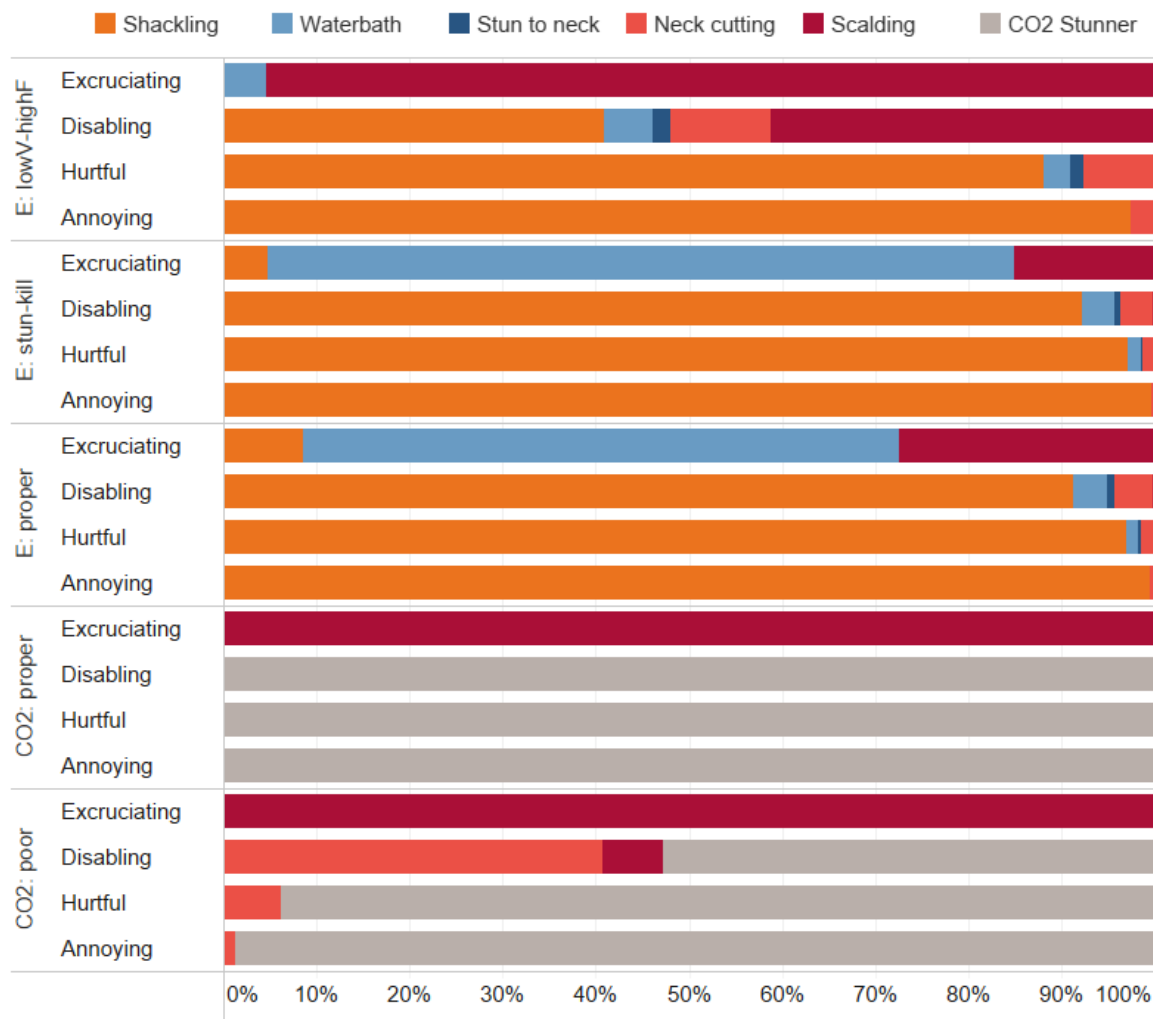


Figure 6. Contribution of each harm **relative** to the total estimated time (scaled to 100%) endured **by the average flock member (i.e. weighted by prevalence)** in the four categories of pain intensity. Here, the absolute size of each colored bar should not be compared across intensities or systems. (E: lowV-highF) low voltage and high frequency stunning parameters are used to reduce the risk of carcass damage, increasing the likelihood of electro-immobilization, (E: stun-kill) stunning parameters are such so that most birds are stun-killed, (E:proper) stunning parameters are appropriately set to achieve effective stunning in as many birds as possible (CO2: proper): multi-stage CO2 system with stunning times and parameters adopted as described by the manufacturers and in the scientific literature, and (CO2: improper): exposure time (or gas concentration) being insufficient to prevent birds from regaining consciousness after leaving the stunner. An interactive version of the figure is available at <https://pain-track.org/broilers/stunning>.

VI. Welfare Impact of a Transition from Electrical Waterbath to Multistage CO₂ systems

Through its effects on the welfare challenges investigated (pain from shackling, inversion, lesions, electrical shocks, fear, breathlessness, ineffective stunning, and the eventual pain from the bleeding and scalding procedures in birds which were ineffectively stunned), a **transition from electrical waterbath systems to properly implemented multi-stage CO₂ systems is expected to substantially reduce the time that the average broiler spends in intense levels of pain (Table 6). Overall, 99 to nearly 100% of the time in Excruciating pain is prevented (excruciating pain is reduced to nearly zero seconds in multi-stage CO₂ systems that are properly implemented), along with 87-90% of the time in Disabling pain. Among other things, millions of birds are expected to be spared from the experience of live (conscious) scalding every year (Table 7).**

Table 6. Estimates of the time (seconds) in pain averted per average bird affected by a transition from electrical waterbath systems to properly implemented multi-stage CO₂ systems, and % reduction (or increase) of the time in pain of each intensity. LowV-highF: low voltage and high frequency stunning parameters are used to reduce the risk of carcass damage, increasing the likelihood of electro-immobilization, Stun-kill: stunning parameters are such so that most birds are stun-killed; Ideal: stunning parameters appropriately set to achieve effective stunning (96-99% effectiveness assumed).

	Time (Seconds) in Pain Averted per Average Broiler: Transition from Electrical Waterbath to Properly implemented CO ₂ systems		
	from lowV-highF systems*	from stun-kill systems	(C) from ideal (hypothetical) systems
Excruciating	6.4 [0.33-12] (- ~100%)	2.1 [0.82-3.4] (-99%)	1.2 [0.5-1.8] (-99%)
Disabling	84 [14-150] (-90%)	60 [14-110] (-87%)	61 [14-110] (-87%)
Hurtful	-11 [-58-39] (+18%)	-17 [-61-25] (+29%)	-17 [-59-26] (+27%)
Annoying	-15 [-46-16] (+ 33%)	-16 [-47-14] (+36%)	-16 [-46-16] (+36%)

* Expected to be typically used where regulation of stunning parameters, or enforcement, is lacking.

Table 7. Estimates of proportion and number of broiler chickens spared from the experience of scalding (while conscious) considering a transition from electrical waterbath stunning systems to properly managed multi-stage CO₂ systems (estimates are based on assumptions described in Tables 4 and 5).

	from lowV-highF systems* to properly managed CO ₂ systems	from stun-kill or ideal electrical systems to properly managed CO ₂ systems
% birds spared	0.08-5.99%	0-0.59%
Approximate no. of birds spared from live scalding per year (examples)	7.5 to 550 million (United States) 5.2 to 350 million (Brazil) 7.7 to 580 million (China)	0 to 44 million (European Union)

* Expected to be typically used where regulation of stunning parameters, or enforcement, is lacking.

In addition to reducing the time spent in more intense levels of pain, in CAS systems where birds are **not** removed from their crates **the possibility of animal abuse is virtually eliminated**. Owing to their random nature, instances of animal abuse other than rough handling and the possibility of fractures during shackling were not quantified in the present analysis, but cases of abuse (involving acts such as throwing and stomping birds) are widely recognized as a major welfare issue in slaughter plants prior to stunning [33]. Still, it is important to consider that, in those cases where bird crates must be manually inspected for the identification, and removal, of dead birds (DOA) prior to the crates' introduction in the gas chambers, the possibility of poor welfare resulting from the human handling of crates will still be present.

These estimates consider multi-stage CO₂ stunning systems such as those from Marel and Meyn. Additionally, available estimates are mostly based on observations in which CO₂ stunning parameters were carefully controlled and monitored [153]. Thus, they are likely to represent the best-case scenario in terms of CO₂ stunning.

Even in properly implemented CO₂ systems, there is, however, a trade-off between (i) the reduction of intense pain due to handling and the by-products of ineffective stunning (e.g. scalding) and (ii) the well-documented aversiveness to the gas and longer duration of suffering until loss of consciousness [33]. Given the relatively long interval between the onset of CO₂ exposure and the gradual loss of consciousness, a longer time in less intense pain (Hurtful and Annoying) is expected (Table 6).

Overall, **these results indicate that carbon dioxide stunning, when properly implemented, is less inhumane than waterbath stunning as currently implemented in commercial conditions**. Although effective electrical stunning is possible if very specific settings are applied (scenario C in Table 6), the variety in parameters and systems used in poultry slaughterhouses, and the focus on speed and minimization of carcass damage makes electrical stunning prone to poor animal welfare. Moreover, low-frequency and high current settings which are favoured on welfare grounds (scenarios B and C in Table 6) can also cause intense muscle contractions and carcass defects that will lead to downgrading [190]. This inherent conflict between effective waterbath stunning and meat quality has led to the widespread adoption of low-current and higher stunning frequencies in the U.S. and other countries, despite the potential for an ineffective stun and substantially poorer welfare [38].

Still, even in European countries, it is not possible to rule out the possibility that, under lax enforcement, staff may alter current settings to improve meat quality. In addition, multiple combinations of frequencies, voltages, waveforms and amount of current applied are legally allowed and some authors have highlighted that some of the parameters currently permitted by EU legislation are not necessarily adequate to induce an effective stunning (epileptiform EEG) [38,191]. Importantly, in all electrical waterbath scenarios the pain and stress associated with the shackling and inversion of live birds (orange bars in Fig.5) is not eliminated.

Welfare Risks of Undersized Equipment and Poorly Managed/Monitored CO₂ Stunning

Advocates of controlled atmosphere systems understand that **it is essential that, rather than being only stunned, birds are kept in the stunner until they are killed by the gas mixture to prevent them from regaining consciousness [33,38,134].**

Nevertheless, concerns have been raised on whether slaughter plants will be willing to leave birds in gas mixtures for a long time [73,189], given the prioritization of fast line speeds and

high throughput rates⁶. The risk of a short exposure is heightened when the line speed exceeds the capacity of the stunner, in which case managers may cut corners and shorten the time birds spend in the chamber [33,152]. As Temple Grandin advises, *“If you get one [CAS stunner], you have to buy a large enough machine so if you expand production, you aren’t tempted to speed up the process. I can’t emphasize it enough that you can’t speed up the process”* [152].

Short exposure is likely to result in large numbers of birds regaining consciousness while shackling, before neck cutting or during bleeding, in which case many could also go into the scald tank in a conscious state. Without regulation and effective enforcement of CAS stunning parameters, the possibility of a short exposure cannot be ruled out - particularly as voluntary compliance has been shown to be ineffective [192]. Shortages of CO₂ (such as those resulting from the disruption caused by COVID containment measures [143]) may also compromise the availability of CO₂ for proper stunning.

Additionally, downtime in poultry slaughter plants due to breakage is also common (ENC, *personal observation*). When lines stop, birds will be ‘stuck’ at any of the stages of the stunning process. Those that left the stunner and were not killed might regain consciousness, and experience the shackling, neck cutting and potentially scalding process in a conscious state.

Other factors such as a reduced concentration of CO₂ in the final stages may also affect the effectiveness of stunning [188]. This is particularly important since compared with electrical waterbath stunning, CAS systems require more maintenance and more skilled staff for the careful and continuous monitoring of the gas mixtures and the calibration of the equipment [33]. This has led some authors to conclude that *“in a poorly managed CAS stunner, there is a point where the induction process may become so poor that live shackling may be less stressful”* [33]. Indeed, as shown in Figure 4, **in such cases the time in Excruciating pain can be longer than that typically associated with electrical waterbath systems where stunning parameters are carefully regulated (stun-kill and ideal electrical waterbath systems) given the relatively high number of birds experiencing the bleeding process (and potentially scalding) in a conscious state.**

Other Critical Assumptions Affecting the Estimates

Many are the uncertainties and knowledge gaps associated with research on stunning methods. There is yet no consensus on the extent to which different behavioural indicators, signs and reflexes can accurately identify loss of consciousness, with a large variation in estimates to time of consciousness even in those findings based on EEG activity (Box 3). Additionally, **many of the relevant and impactful CAS research studies has been funded by CAS systems manufacturers.** This is the case of CO₂ multistage systems research commissioned and funded by Meyn Food Processing Technology [153] and Praxair Inc [151], on which the present estimates are also based.

Additionally, as discussed throughout this document, many are the research gaps involving the study of the affective experiences of birds exposed to the many welfare offenses encountered at slaughter plants. One research gap that seems particularly relevant relates to the implications of fear and the potential for fear-induced analgesia in chickens (Box 2). **Further studies are needed to determine the extent to which pain is suppressed in situations of**

⁶ In the U.S., line speed is regulated by the USDA based on Food Safety standards [23] and it is only limited by federal sanitation laws (meaning the line is only halted upon the identification of some potential contamination); other attempts at regulating line speed have been blocked by interference from different branches of government and congress [24].

extreme fear. Should this possibility hold, it would have major implications for the understanding and quantification of pain during stunning and slaughter.

Likewise, assumptions on the extent to which birds are fearful prior to unconsciousness are also critical. Here, we assumed that fear prior to stunning is more intense in waterbath systems than in multistage CO₂ systems given the rough handling and inversion of birds in the former case (along with observation of wing flapping and other signs of struggle), and the apparent lack of signs of fear and distress in multistage systems (in carefully controlled trials). However, research in humans and other mammals shows that CO₂ is associated with a primordial fear of suffocation, triggered through the detection of carbon dioxide and acidosis by the amygdala, an extremely ancient structure also present in birds [187]. Research has shown that fear and panic in humans and rats are suppressed with the administration of antianxiety drugs [186]. Yet the effect of such drugs, or those known to relieve breathlessness in humans such as inhaled furosemide, on chickens' responses to CO₂ have not been explored.

Therefore, the estimates presented here represent only a first attempt at quantifying the time spent in pain of different intensities deriving from the main methods for the commercial slaughter of broilers, and should be revised as knowledge advances. The need for independent research in the areas discussed cannot be stressed enough.

Strategic Risks associated with a Transition to CO₂ stunning systems

Our results indicate that, if properly implemented, CO₂ stunning systems are less inhumane than electrical waterbath systems as currently implemented (i.e. set to reduce carcass damage, or to stun-kill animals), reducing the time spent in the more intense levels of pain.

There are market forces that can support the transition, as once implemented, CO₂ stunning offers improved meat quality owing to the reduced stunner-related carcass damage⁷ [193,194], making future investments in other systems less likely. When compared to other gases, CO₂ also seems to have a competitive edge for bigger operations as CO₂ is inexpensive and more readily available than other gases [154], and runs at lower operating costs [74].

Still, other stunning systems, not investigated here, are in general thought to be less aversive than carbon dioxide [144]. One such alternative is the use of inert gases like argon or nitrogen. Stunning with inert gases has shown reduced signs of aversion compared to CO₂, although convulsions can be vigorous, affecting meat quality [163]. Another alternative is the use of Low Atmospheric Pressure Stunning (LAPS), a method in which birds are exposed to gradual decompression that reduces oxygen levels to less than 5%. LAPS was approved for use in the EU in 2018 after legislators deemed it 'not worse' than previously approved methods [195], though research on the welfare benefits of LAPS is still not conclusive.

VI. Priorities in the Alleviation of Suffering: comparing extreme pain endured at slaughter and at other life stages

Many argue that **preventing or alleviating the most extreme forms of pain and suffering is an ethical priority, of greater urgency than alleviating less severe states of suffering**, regardless of how long they last [196–198]. We concur with this view. That is one of the main reasons why,

⁷ In this sense, EU processors might be particularly inclined to consider a transition. In the U.S., carcass damage is prevented through the use of low voltage and high frequency stunning parameters, hence there has been less interest in CAS systems [143].

in developing the [Cumulative Pain Framework](#), we opted for keeping the time spent in pain of different intensities disaggregated. It is not unreasonable to assume that some intensity categories are fundamentally different, in that no time in Annoying, Hurtful or even Disabling pain could ever outweigh even a few minutes, or seconds, of torture-like (Excruciating) pain (intensity definitions in Box 1). Keeping estimates of the time spent in pain of different intensities disaggregated is thus essential to enable the identification and pursuit of different priorities, including the relief of extreme forms of suffering, such as those endured at the slaughter plant [82].

The stunning of broiler chickens in electrical waterbath systems involves, for some individuals, experiences such as bone breakage during shackling, electrocution that achieves only immobilization (but not loss of consciousness) and live scalding, one of the worst forms of torture ever devised. In this chapter, we estimate that these experiences translate into over 6 seconds of Excruciating pain for the ‘average broiler’ (see Box 3 for a definition) in typically used electrical waterbath systems (low voltage, high frequency) and, more importantly, into up to 120 seconds of Excruciating pain for the individual birds actually enduring these experiences. Alleviation of this suffering, however, is possible: **a transition to multistage CO₂ stunning systems is estimated to prevent nearly all Excruciating pain if (but only if) these systems are properly implemented and managed.**

But how does a transition to multistage CO₂ stunning compare to other interventions aimed at preventing extreme forms of acute suffering at other life stages? **The potential for interventions — other than stunning reforms — to prevent extreme forms of suffering depends on the extent with which one agrees that conditions other than those experienced in the slaughter plant also cause Excruciating pain in their victims.** For example, a transition from fast- to slower-growing broiler breeds was estimated to prevent, for the ‘average broiler bird’, 25 (5-45) seconds of Excruciating pain (Chapter 7). This reduction in the time in Excruciating pain emerges from the lower likelihood of fatal cases of ascites and severe sepsis in slower-growing birds. Fatal victims of ascites are estimated to endure about half an hour (Chapter 3) of Excruciating pain due to the highly stressful nature of the breathlessness produced by the accumulation of fluid and pressure in the abdomen in the moments preceding death⁸. Additionally, given their poorer immune responses [79], fast-growing birds have a heightened susceptibility to infectious diseases, hence higher likelihood of severe sepsis. Severe sepsis is often accompanied by major organ failure, hemorrhages on the heart, liver, kidneys, muscles and serous membranes which are often swollen and haemorrhagic. Muscles are also *‘wasted away and broken down because of changes in muscle metabolism’* [199]. In humans, pain from severe sepsis is described as extreme (*“I feel like I might die”; “My sepsis felt like my body was on fire, burning from the inside out”*) [200,201]. To the extent that these instances of Excruciating pain suffered at the farm level can be compared with those endured by some individuals at slaughter (particularly the experience of live scalding), a transition to slower-growing breeds would have a greater potential of alleviating extreme suffering (Table 8).

⁸ A video of a bird with ascites - still in a Disabling state - is available here: <https://youtu.be/IMh4zjv-FF8>

Table 8. Estimates of the time in pain averted per average bird affected by welfare reforms for broiler chickens

	Time in Pain Averted per Average Broiler		
Pain Intensity	Transition from electrical waterbath (set to reduce carcass damage) to proper multi-stage CO2 stunning	Transition from electrical waterbath (stun-kill) to proper multi-stage CO2 stunning	At the farm level: adoption of slower-growing breeds*
Excruciating	6.4 [0.33-12] seconds	2.1 [0.82-3.4] seconds	25 [5 to 45] seconds
Disabling	84 [14-150] seconds	60 [14-110] seconds	35 [18 to 53] hours
Hurtful	-11 [-58-39] seconds	-17 [-61-25] seconds	95 [-67 to 260] hours

*growing at 45-46 g/day

Likewise, it is also possible that the pain endured by individuals experiencing multiple fractures during the process of depopulation (when they are caught, carried and roughly placed into crates) may also reach an Excruciating intensity [202]. During depopulation, commonly broken bones include hip and legs (humerus, ischium and pubis), keel and wings. Different from other bone fractures endured during growth, the pain and discomfort resulting from pre-transport fractures is likely exacerbated by the traumatic mode of fracture and the inversion and extremely rough handling of the birds [203,204]. For laying hens, a 5% probability that the pain from multiple fractures during depopulation is of an Excruciating intensity translates into a total of 4-16 seconds of Excruciating pain over the course of the depopulation process (which lasts from 1.35 to 5.5 minutes from the moment of catching until placement into the transport crates) [202]. Intense pain may also persist during the long journey to the slaughter house. A similar situation may also occur in the case of broilers, who are also known to endure multiple fractures during the depopulation process [17,54]. Should interventions aimed at improving the depopulation process be implemented, the potential benefits in the reduction of these instances of acutely intense pain would be also substantial.

Naturally, this discussion does not consider differences in the tractability of the different types of interventions. Nor does it take into account other flow-through effects of the interventions in terms of public attitudes regarding farm animal welfare or demand for animal-sourced products. Instead, it is aimed at reducing uncertainty regarding important hotspots of intense suffering in the production chain, and the direct welfare benefits expected should any of the interventions be adopted.

Main Findings

- From the moment of placement in the stunning/slaughter system (i.e. excluding lairage time) until loss of consciousness, the average broiler is expected to spend 0.02 [0.01-0.03] seconds in Excruciating pain and 8.82 [5.4-12.2] seconds in Disabling pain in properly managed multi-stage CO₂ systems, compared to
 - (i) 2.18 [0.9-3.5] seconds in Excruciating pain and 69 [23-115] seconds in Disabling pain in electrical waterbath systems where birds are fatally electrocuted (stun-kill systems typically used in the EU);
 - (ii) 6.4 [0.5-12.3] seconds in Excruciating pain and 93 [25-161] seconds in Disabling pain in electrical systems set to reduce carcass damage (low voltage-high frequency, typically used in the U.S. and countries lacking stunning regulation); and
 - (iii) 1.19 [0.6-1.8] seconds in Excruciating pain and 70 [24-115] seconds in Disabling pain in electrical systems that ensure the highest possible (96%) effectiveness of stunning by electronarcosis.
- Most of the time in Excruciating pain derives from the experience of live scalding (expected to affect very different proportions of birds in each system) and, to a lesser extent, from electro-immobilization and fatal electrocution in electrical waterbath systems. Disabling pain is associated (though not continuously or exclusively) with the pain from the compression of injured legs during shackling, wing fractures, pre-stun shocks, electro-immobilization, fatal electrocution and the incision of vessels and carotids. In CO₂ stunning, Disabling pain emerges from the possibility that the sensation of breathlessness and aversiveness to CO₂ reach a Disabling level at certain CO₂ concentrations.
- A transition from electrical waterbath systems to properly implemented multi-stage CO₂ systems is expected to substantially reduce the time the average broiler spends in acutely intense pain. Overall, 99% to nearly 100% of the time in Excruciating pain during stunning could be prevented, along with 87-90% of the time in Disabling pain.
- Such a transition would also spare 7.5 to 550 million birds in the United States and up to 44 million birds in the European Union from the experience of live scalding every year.
- In CAS systems where crates are not opened prior to stunning (for the identification/removal of birds dead on arrival at the slaughterhouse or placement of birds into stunning units), the possibility of animal abuse is virtually eliminated.
- However, there is a trade-off between a reduction of intense pain with the use of CO₂ stunning systems and an increase in the duration of (milder) pain given the aversive nature of CO₂ prior to loss of consciousness. In general, a 27-36% longer time in Hurtful and Annoying pain is expected in multi-stage CO₂ systems compared to electrical waterbath systems.
- The expected welfare benefits of CO₂ stunning systems are lost if exposure times or gas concentrations are insufficient to ensure that birds do not regain consciousness after leaving the gas stunner. Slaughter plants may not be willing to leave birds in the stunner for a long time given their prioritization of speed and high throughput rates. Short exposure time is likely to result in a large fraction of birds regaining consciousness, who could thus experience the pain associated with neck cutting, bleeding and scalding. In such cases, the estimated time in intense pain will be longer [3.2 (0.03-6.37) seconds in Excruciating pain and 14.5 (5.65–23.3) sec in Disabling pain than that typically associated with 'properly set and managed' electrical waterbath systems.
- It is critical that reforms focusing on the implementation of CO₂ stunning ensure the adoption of mechanisms to guarantee that birds are killed in the gas stunner.
- Market forces support the transition, as once implemented CO₂ stunning is associated with reduced carcass damage. Still, other gas stunning systems not investigated here (e.g. inert

gases) are thought to be less aversive to poultry than carbon dioxide, yet a transition to CO₂ stunning makes future investment into other systems less likely.

- Preventing or alleviating the most extreme forms of pain and suffering, including the acutely intense and concentrated states of pain endured at slaughter plants, is an ethical priority, often viewed as being of greater urgency than alleviating less severe states of suffering. The potential for interventions — other than stunning reforms — to prevent extreme forms of acute suffering depends on the extent with which one concurs that conditions other than those experienced in the slaughter plant also cause Excruciating pain in their victims. Among these conditions are fatal cases of ascites, associated with acute breathlessness and visceral pain produced by the accumulation of fluid and pressure in the abdomen in the moments preceding death, as well as cases of extreme pain from severe sepsis, which are accompanied by major organ failure, hemorrhages on the heart, liver, kidneys, muscles and serous membranes (in humans, severe sepsis is often equated to severe burning, e.g. '*my sepsis felt like my body was on fire, burning from the inside out*'). To the extent that these instances of extreme pain at the farm can be compared with those endured by some individuals at slaughter (particularly the experience of scalding), other interventions could have a greater potential of alleviating extreme suffering (e.g. the transition from fast- to a slower-growing breed reaching a slaughter weight of 2.5 Kg at 56 days is expected to prevent 35 [18 to 53] hours of Disabling pain, 95 [-67 to 260] hours of Hurtful and 25 [5 to 45] seconds of Excruciating pain for every bird affected by this intervention). Naturally, these estimates do not consider differences in the tractability of the different interventions or their flow-through effects in terms of public attitudes regarding farm animal welfare or demand for animal-sourced products.

Box 5. Research Gaps and Opportunities Identified

- Data is extremely scarce on the proportion of birds not effectively stunned and still conscious during bleeding and prior to entering the scald tank in electrical waterbath systems under typical commercial conditions, particularly in non-European countries. To our knowledge, there is also no data available on the proportion of non-stunned birds that have their arteries improperly severed. Further studies in abattoirs, particularly in major chicken producing countries, are very much needed to determine these figures more accurately.
- No data is available on the proportion of birds that typically receive pre-stun shocks in electrical waterbath systems, or the number of shocks typically received by them.
- There are only two publications that report on the behavioral responses of broilers exposed to multistage CO₂ systems in commercial conditions, in which the results are disaggregated for each stunning stage. Also, there have been no measurements of the proportion of birds regaining consciousness depending on the time of exposure to CO₂ in commercial systems.
- Many of the relevant and impactful CAS research has been funded by CAS systems manufacturers. Further independent research is greatly needed.
- Further studies are critically needed to determine the extent to which pain is suppressed in situations of extreme fear (fear-induced analgesia is known in many species, and may also be present in chickens). Should this possibility hold, it would have major implications for the understanding of pain and suffering, particularly during the stunning and slaughter process.
- Whether the neural circuitry for fear in birds is activated by exposure to hypercapnic gases has not yet been investigated. Since in mammals fear is triggered through the detection of carbon dioxide and acidosis by the amygdala, an extremely ancient structure also present in birds, a similar mechanism is most likely to be present in chickens.
- Research has shown that fear and panic in mammals are suppressed with the administration of antianxiety drugs. Yet the effect of such drugs, or those known to relieve breathlessness in humans such as inhaled furosemide, on chickens' responses to CO₂ have not been explored.
- There is yet no consensus on the extent to which different behavioural indicators, signs and reflexes can accurately identify loss of consciousness, with a large variation in estimates to time of consciousness even in those findings based on EEG activity. Further research is also needed to determine the extent to which convulsions after loss of posture can occur in conscious birds.
- Ineffective stunning with CAS is likely to occur if gas concentrations are not properly adjusted or exposure time is insufficient, in which case a large fraction of birds may regain consciousness and experience the pain from neck cutting and scalding. This has led to concerns on whether slaughter plants will be willing to leave birds in gas mixtures for a long time, given the prioritization of speed. An understanding on the likelihood that compliance with proper CO₂ stunning parameters will be achieved is thus greatly needed.

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