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Comparison of Methods for Individual Killing of Broiler Chickens: A Matter of Animal Welfare and On-Farm Feasibility

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The humane killing of individual broiler chickens on-farm requires a minimum of suffering. In this regard, rapid and irreversible loss of consciousness are important determinants. This can be verified by cerebral and spinal reflexes. Also, on-farm feasibility determines whether producers will apply the method. The aim of the study was to compare the effectiveness and animal welfare impact of two different methods for killing individual broilers of varying ages (2, 4, and 6 weeks): manual cervical dislocation (CD) and captive bolt (CB). The evaluation of CD and CB was based on effectiveness and on time to onset (convulsions) or cessation (pain response, pupillary light reflex, convulsions, heartbeat) of non-invasive indicators. In addition, a pilot study was conducted on-farm to assess the feasibility of two alternative methods, CB and nitrogen gasification (N2), and to survey farmers' opinions on them. The onset of convulsions was almost immediate for both methods in the first study. No differences between CD and CB were observed for the cessation of pain response for chickens at age of 2 weeks (5.0 and 7.5 s, respectively) and 6 weeks (14.0 and 14.1 s, respectively). However, at 4 weeks a longer pain response was measured after CD (11.3 s) than after CB (4.7 s). For the three age categories, the pupillary light reflex disappeared later after CD (54.9 - 80.7 s) compared to CB (8.3 - 13.7 s). The same was observed for cessation of convulsions in 2- and 6-week-old chickens (185.3 and 172.0 s for CD and 79.0 and 82.9 s for CB). This suggests that brain death occurred faster after CB compared to CD. No difference between the methods was found for the cessation of the heartbeat. After the pilot study, the producers preferred N2 over CB in terms of animal-friendliness, time-efficiency, ease of use, and effectiveness. However, both methods were found rather expensive and required some experience. CB and N2 are good killing alternatives to CD due to rapid and irreversible insensibility. However, more information and support for chicken producers will be needed for these to become routine killing methods.

Keywords: broiler chicken, killing, on-farm, cervical dislocation, captive bolt, nitrogen gassing

INTRODUCTION

The protection and welfare of broiler chickens in the European Union (EU) is covered in Council Directive 2007/43/EC (European Union, 2007). Birds must be inspected at least twice a day, with special attention given to signs indicating health or welfare problems. In the poultry industry, sick individuals rarely receive medical treatment with the aim of recovery. Instead, they are killed to avoid further or future suffering, avoid possible contamination of other flock members, avoid treatment costs, or minimize production losses (Berg, 2009; EFSA, 2019). Flemish chicken producers inspect their flock according to the legal standard (2.2 times per day on average) and they indicated that lameness, a broken leg, and nervous system problems were the main reasons for killing an individual bird (Watteyn et al., 2020). On-farm killing can be applied to any type of poultry of any age, but there are restrictions in the EU on the use of methods with regard to the maximum body weight of the animal and the number of birds that may be killed by a person per day. Moreover, not all methods are suitable for killing individual birds (**Table 1**). Manual cervical dislocation (CD) by stretching and twisting the neck (HSA, 2016) is by far the most common method for killing broilers on-farm (Sparrey et al., 2014; Watteyn et al., 2020), but many alternative methods exist. Mechanical methods, except for maceration, are the most feasible options. More specifically, the captive bolt method (CB) is characterised by brain damage and consequently a rapid loss of consciousness. Most of those techniques are independent of physical strength and can therefore be used for many repetitions or heavy animals. Although a lethal injection with pharmaceuticals like barbiturates is ideal for individual killing, it will not often be used due to the high cost of the product and the veterinarian (the only profession authorized to use such methods in the EU). Electrical and modified atmosphere killing methods are used in slaughterhouses or for depopulation, but with some adaptation of the equipment (e.g., using gas in closed containers), it can also be used for killing individual birds on-farm (Boyal et al., 2020; Jacobs et al., 2021). Carbon dioxide is a well-known gas used for depopulation in chicken stables. However, poultry show aversive reactions to carbon dioxide inhalation (Raj, 1996), whereas nitrogen (N2) is better tolerated. Furthermore, using N2 is more advantageous in terms of shortening the time to death as compared to carbon dioxide (Gurung et al., 2018). The body weight limits (max 3 kg or 5 kg) apply only to mechanical methods (**Table 1**). Consequently, this is less relevant to broiler chickens as they rarely weigh more than 3 kg. However, it is not known if each method is equally feasible for any age, and thus any body weight.

The European Council Regulation 1099/2009 stipulates that on-farm killing of sick and injured birds must be done humanely and that the level of suffering ought to be minimized (European Union, 2009). The latency to irreversible insensibility is an important feature of the welfare impact of a killing method. Some methods, such as maceration or CD, cause death while the animal is still conscious, whereas other methods, such as CB or atmospheric killing, induce loss of consciousness prior to death (EFSA, 2019). Brain death is defined as irreversible loss of brain function, including the brain stem (Wijdicks, 1995). The gold standard for assessing brain function is the use of electroencephalogram (EEG) for monitoring cerebral cortex activity (Firsching et al., 1992; Knudsen, 2005; Lowe et al., 2007). Both the state of consciousness and brain death can be

TABLE 1 | Killing methods allowed for poultry, with indication of cause of death, restrictions on using the method regarding maximum body weight of the birds (BW), or maximum number of birds that may be killed per day per person, and whether or not the method is suitable for killing individual birds (as opposed to killing of entire flocks) (European Union, 2009; HSA, 2016; EFSA, 2019).

	Cause of death	Restrictions	Individual killing
Mechanical			
Maceration	Brain damage	Chicks < 72h	No
Manual CD	Spinal cord damage Cerebral ischemia	< 3 kg BW < 70 animals/day/person	Yes
Mechanical CD	Spinal cord damage (Cerebral ischemia)	< 5 kg BW	Yes
Percussive blow to the head	Brain damage	< 5 kg BW < 70 animals/day/person	Yes
Penetrative CB device	Brain damage		Yes
Non-penetrative CB device	Brain damage		Yes
Firearm with free projectile	Brain damage		
Electrical			
Electrical stunning – head-to-body	Fibrillation and stopping of heartbeat		Yes
Modified Atmospheres			Yes, only when it can be done in a closed container
Gas – CO_2	Hypercapnia - Hypoxia		
Gas – CO_2 + inert gas	Hypercapnia - Hypoxia		
Gas – inert gas	Hypoxia		
Gas – CO	Hypoxia		
Gas – CO + other gas	Hypoxia		
Chemical			
Lethal injection	Depression of CNS	Application by veterinarian (in EU)	Yes

CD, cervical dislocation; CB, captive bolt; BW, body weight; CNS, central nerve system; EU, European Union.

recorded. An issue when measuring brain activity during on-farm experiments is environmental disturbances, which can lead to noise on the EEG (Erasmus et al., 2010c). Blood flow to the brain is essential for brain activity. Post-mortem examination of the brain to identify subcutaneous haemorrhages at the neck in case of CD, or submeningeal haemorrhages in case of CB, may indicate interruption of the blood flow.

In addition, testing cerebral and spinal reflexes such as pupillary light reflex, corneal reflex, pain reflex, and indicators like convulsions, rhythmic breathing, and heart rate can reflect brain activity (Erasmus et al., 2010a; Erasmus et al., 2010b; Sandercock et al., 2014). The nuclei and tracts of the oculomotor nerve (cranial nerve, CN III), involved in the pupillary light response, are located in the upper part of the brain stem. By contusion of the brain, those tracts and nuclei might be damaged (Orosz and Antinoff, 2016), suggesting that the pupillary light response is a reliable indicator of complete insensibility (Croft, 1961). Both corneal and pain reflexes are triggered by the trigeminal nerve (CN V), which arises from the medulla oblongata (Orosz and Antinoff, 2016). The medulla also contains the reflex centre for respiration and the nuclei of the vagus (CN X), which is responsible for the parasympathetic innervation of the heart. Accordingly, damage to the medulla will result in a lack of corneal and pain reflex, rhythmic breathing, and an increase in heart rate (Orosz and Antinoff, 2016). The pain reflex is absent when birds are unconscious (Sandercock et al., 2014). The corneal reflex is the last reflex to disappear but has been used to assess insensibility on-farm (Wotton and Sparrey, 2002). Also, the loss of spinal reflexes indicates brain damage (Orosz and Antinoff, 2016). Poultry first show a phase of clonic muscle movements (wing-flapping), followed by tonic movements (stretching of wings and legs), which are generated by lesions to the higher upper motor neurons in the cerebral cortex (Erasmus et al., 2010c; Orosz and Antinoff, 2016). Dawson et al. (2009) demonstrated a positive correlation between the cessation of those convulsions and an iso-electric EEG. Hence, this can be used as a sign of brain death.

The costs and on-farm feasibility are important criteria of a killing method as well, as they determine whether incentives will be needed to ensure uptake by the broiler industry. Only one study investigated the on-farm feasibility of killing methods (Martin et al., 2018). Therefore, it is important to compare different methods for killing individual poultry with regard to their humaneness and on-farm feasibility, as so far only a little information is available.

The main objective of this study was to compare the effectiveness and animal welfare impact of two different methods for killing individual broilers of varying ages: the most used method, manual CD, and an alternative method, CB. The results were compared with those of a previous study where N2 was tested (McKeegan et al., 2013). In addition, a pilot study was performed to assess on-farm feasibility and farmer opinion of two alternative killing methods, CB and N2.

MATERIAL AND METHODS

Ethical Approval

All animal experiments were approved by the ethical committees of Flanders Research Institute for Agriculture, Fisheries and Food (2017/296) and Ghent University (2018/10), and by the Department of Environment – Animal Welfare of the Flemish Government.

Effectiveness and Animal Welfare Study

Animals and Experimental Design

The study was performed on broilers (Ross 308, both sexes) from the same flock, housed at a local commercial broiler farm, using three age categories (2, 4, and 6 weeks old). All experiments were conducted on-farm, outside of the barn where the broilers were housed. For each age category, healthy broilers were taken at random out of the house, carried upright, and placed in a confined box. The killing method, either manual CD or a CB system, was randomly assigned to a box (**Table 2**). Manual CD was conducted according to the procedure explained by the Humane Slaughter Association (HSA, 2016). The operator (trained researcher) held both broiler legs in one hand while holding the head just behind the skull with the other hand. Cervical dislocation was achieved by stretching and turning the head. For the other method, a Zephyr-EXL pneumatically powered (by air compressor) non-penetrating CB (Bock Industries, Philipsburg, USA) device was used by the same operator, in accordance with user manual instructions. In short, while the chickens were restrained upside down in a cone, the CB was placed on the chicken's head, in the middle between the eyes and ears. One discharge was given to the bird. The recommended air pressure (8.3 bar) was lowered to 5.5 – 6.6 bar (variation in pressure due to handling) to minimise the loss of blood through the eyes and nostrils. A preliminary test showed that the recommended pressure caused a lot of bleeding, which is undesirable from a biosafety point of view.

Measurements of Non-Invasive Indicators

The latencies to loss of consciousness, brain, and clinical death were assessed using five non-invasive indicators. Specifically, the length of time (in s) was recorded from the moment the killing manipulation was completed to the moment when the pain response (withdrawal of head when pinching the comb with fingernails of researcher) and the pupillary reflex (constriction of pupil when shining a light into the eye) ceased. In addition, the onset (start of wing flapping) and ending of convulsions (complete muscle relaxation), and the absence of a rhythmic heartbeat (measured with stethoscope) were registered. The indicators were assessed by two trained researchers. Researcher 1 assessed, in the following order: (1) pain response, (2) pupillary light reflex, and (3) heartbeat. Researcher 2 assessed (1) onset and (2) ending of convulsions. The assessments by researcher 1

TABLE 2 | Experimental design of Effectiveness and Animal Welfare Study.

Age	2 weeks	4 weeks	6 weeks
Killing method – CD	n=12	n=20	n=20
Killing method – CB	n=6	n=10	n=10
Body weight (kg, mean ± SD)	0.44 ± 0.05	1.44 ± 0.15	2.70 ± 0.30

Two killing methods, manual cervical dislocation (CD) and captive bolt system (CB), were tested on broilers of 2, 4, and 6 weeks old. Non-invasive indicators were measured after both manual CD and CB. SD, standard deviation.

were repeated in the same sequence each time until the indicator stopped.

If an animal remained sensible after 1 min (positive pain reflex or absence of convulsions), the killing method was considered ineffective, and the animal was euthanized by injecting sodium pentobarbital (450 mg/kg BW) in the brachial (wing) vein.

Post-Mortem Examination

All chickens killed in this experiment (n=52 for CD, n=26 for CB) were examined post-mortem by a trained pathologist. After CD, the birds were evaluated on the presence/absence of skin lesions (neck), presence/absence of subcutaneous haemorrhages (neck), lesions to cervical vertebrae (dislocation, fracture, or contusion), presence/absence of spinal cord damage and location of dislocation, contusion, and spinal cord damage. After CB, the birds were evaluated on the presence/absence of skin lesions (head), presence/absence of external bleeding (eyes, nostrils, mouth), presence/absence of subcutaneous haemorrhages (head), cranial fractures (scoring), presence/absence of brain damage (cerebrum and cerebellum), submeningeal haemorrhages (location and scoring). The severity of submeningeal haemorrhages and skull fractures was assessed using the scoring system of Veltri and Klem (2005) and Erasmus et al. (2010b) (**Table 3**). Based on the result of the post-mortem examination, it was determined whether the killing method had been performed properly and the intended injuries had led to a quick death. A CD killing was considered accurate if the spinal cord was completely ruptured, regardless of a complete dislocation or a fracture of the cervical vertebrae. A CB killing was considered accurate if brain damage (cerebrum or cerebellum) or a submeningeal haemorrhage (at cerebrum or cerebellum) scored > 1.

Statistical Analyses

The time measurements of the non-invasive indicators were analysed using a linear model with indicator as the dependent variable and killing method (CD or CB), age (2, 4, or 6 weeks), and their interaction, if statistically significant, as independent variables. The onset of convulsions was not statistically analysed as this indicator was not normally distributed and common non-parametric tests were not possible due to the many similarities in the data. The reported p-values are based on the ANOVA type III F-test, followed by *post-hoc* test with a Tukey correction for multiple comparisons. To measure the degree of the relationship between the non-invasive indicators a Pearson r correlation test was performed. All analyses were performed using R version 3.6.0 (R Core Team, Vienna, Austria). A value of $P < 0.05$ was considered significant.

TABLE 3 | Scoring system for evaluating the severity of skull fractures (from 0 to 4) and submeningeal haemorrhages (from 0 to 5) after killing with captive bolt method (adapted from Veltri and Klem, 2005 and Erasmus et al., 2010b).

Score	Definition
Skull fractures	
0	No fractures, intact skull
1	Hairline fractures, no separation of bone
2	1 or 2 complete, fully separated fractures or a single depressed fracture
3	3 to 5 complete fractures, more than just a single depressed fracture
4	>5 complete fractures, fully fragmented skull
Submeningeal haemorrhages	
0	None
1	Some bleeding, < 25% of surface area
2	Bleeding between 26 – 50% of surface area
3	Bleeding between 51 – 75% of surface area
4	Severe bleeding, 76 – 99% of surface area
5	Completely covered

Small Scale Feasibility Study

Two broiler chicken producers were self-selected from a previous study (Watteyn et al., 2020). Both producers used manual CD as their standard method of killing individual animals. In this experiment, they used alternative methods in their daily management (selection of sick or injured broilers). A researcher trained the chicken producers to use both methods. The two alternative killing methods were each tested for a 2-week period at the end of a batch, in one house. All broilers were Ross 308, both sexes with ages between 4 and 6 weeks. They were all housed on a commercial farm, according to general management housing conditions. CB was performed by Zephyr-EXL (Bock Industries, Philipsburg, USA) following the user manual with some adaptation concerning the pressure (see Study 1). The other method was N2-filled high expansion foam (RASA, Putten, The Netherlands). The chickens were placed in a container (69*53*50 cm). After closing the container, it was filled with high expansion foam (diameter of 15 mm) containing nitrogen, at a filling rate of 500 L/min. The birds were immersed in the foam and inhaled nitrogen instead of oxygen resulting in anoxia followed by death. Producers have used alternative methods to CD to cull chicks. A total of 84 animals were killed by CB and 173 by N2. When producers were unsure if the killing was effective, they were instructed to kill the bird by manual CD after application of CB or N2. The number of those killed birds was noted and considered as inaccurate killings. A random subsample of chickens killed by CB (n=36) was examined post-mortem, using the same methodology as described for Study 1. This was not performed in chickens killed by N2, as it was assumed that there would be no visible lesions.

After the testing phase, the producers were asked their opinion about the two alternative killing methods. They had to quantify 10 properties (animal-friendliness, time-efficacy, ease of execution, effectiveness, feasibility to perform inside the chicken house, cost-efficacy, operator safety, maintenance requirements, risk of fatigue, and perceived preference of the veterinarian) on a 5-point scale: completely agree (5), rather agree (4), neutral (3), rather disagree (2), and completely disagree (1), *via* a paper survey. The respondents could also list any advantages and disadvantages of each method in an open-ended question.

RESULTS

Effectiveness and Animal Welfare Study

The success rate was 100% for each method for all ages. The non-invasive indicator responses are shown in **Figure 1**. The latency

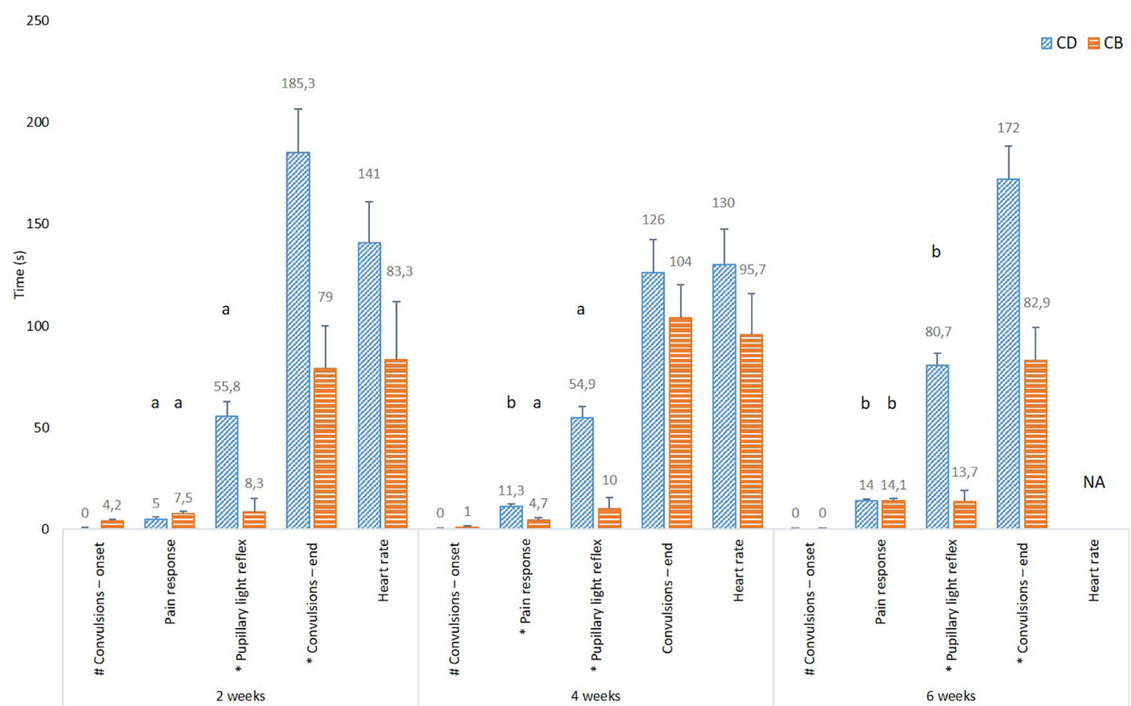

FIGURE 1 | Evaluation of two killing methods, manual cervical dislocation (CD) and captive bolt (CB) in broiler chickens of 2, 4, and 6 weeks old. Mean (SE) duration in s of onset (convulsions – onset) or cessation (pain response to comb pinch, pupillary light reflex, convulsions – end, heart rate) of non-invasive indicators. Time measurements started from the moment the killing manipulation was completed. * indicates significant difference observed for method within age (P<0.001). a, b indicates significant differences observed for age within a method (P<0.05). #, the results were not statistically analyzed as the indicator was not normally distributed and also non-parametric tests were not possible due to too similar values. NA, measurements of the heart rate were not possible at 6 weeks of age due to thickness of the breast muscles. Convulsion-onset and pain response are indicators for insensibility, convulsion-end is an indicator of brain death, whereas pupillary light reflex can be an indicator for both. Heart rate is an indicator of clinical death.

until loss of the pain response was similar for both killing methods for chickens at ages of 2 and 6 weeks. However, at 4 weeks a longer pain response was measured after CD (11.3 s) than after CB (4.7 s; $P < 0.0001$). At all three ages, the pupillary light reflex disappeared faster after CB (8.3 - 13.7 s) compared to CD (54.9 - 80.7 s; $P < 0.0001$). The convulsions in 2- and 6-week-old chickens ended faster after CB compared to CD (respectively 79.0 and 82.9 s for CB, and 185.3 and 172 s for CD; $P < 0.001$) but did not differ for 4-week-old chickens ($P = 0.35$). The onset of convulsions was almost immediate for both methods, although there was a short delay after CB at the age of 2 weeks (4.2 s). There was no difference between both methods in the latency until the cessation of the heartbeat at 2 ($P = 0.11$) and 4 weeks ($P = 0.21$). At 6 weeks, it was impossible to hear the heartbeat due to the thickness of the breast muscles. For CD, there were age differences for the pain response (between 2 and 4 weeks, $P < 0.001$, and 2 and 6 weeks, $P < 0.0001$) and for the pupillary light reflex (between 2 and 6 weeks, $P = 0.02$, and 4 and 6 weeks, $P < 0.01$), with the youngest birds having a faster loss of response. For CB, there were no age differences apart from a longer pain response in week 6 compared to weeks 2 and 4 ($P < 0.001$ and $P < 0.0001$, respectively).

Positive correlations were found for the loss of pupillary light reflex with the ending of convulsions ($R^2 = 0.69$, $P < 0.001$), and cessation of heartbeat ($R^2 = 0.53$, $P = 0.009$). Also, ending of convulsions was positively correlated with cessation of heartbeat ($R^2 = 0.62$, $P = 0.002$).

The effectiveness of the killings was assessed by post-mortem examinations (**Tables 4**, **5**). All birds killed with CD showed a dislocation between the skull and the first cervical vertebra combined with complete disruption of the spinal cord, and damage to the blood vessels (arteria and veins) which resulted in subcutaneous haemorrhages at the neck. No skin lesions were found. This indicates that the killings by CD were accurate. After CB, half of the birds had skin lesions at the head, caused by the impact of the bolt. Both in young and older chickens, external bleedings were found. The CB killing was considered accurate for 24 out of 26 chickens. One chicken killed by CB had no fractures, macroscopic brain damage, or submeningeal haemorrhages. Another chicken showed a hairline fracture with slight damage to the cerebellum combined with a small haemorrhage. For those two birds, kills were deemed unsuccessful.

Small Scale Feasibility Study

To evaluate the effectiveness of the producer-performed killings, 36 chickens killed by CB underwent post-mortem examinations (**Table 4**). Those chickens had a mean BW of 1.04 kg

TABLE 4 | Summary of the post-mortem examination after cervical dislocation (CD) in Effectiveness and Animal Welfare Study, with the prevalence (in % and ratio) of injuries specific for CD, and decision on the accuracy of execution of the killing method.

CD (n=52)	Prevalence (% – n/52)
Skin lesions (neck)	0.0 0/52
Subcutaneous haemorrhages (neck)	100.0 52/52
Lesions to cervical vertebrae	100.0 52/52
- *dislocation**	*100.0 52/52*
- *fracture or contusion*	*0.0 0/52*
Spinal cord damage	100.0 52/52
Accurate execution	100.0 52/52

All dislocations were located between skull and first cervical vertebrae.

(range: 0.31 to 1.96 kg). Only 5 out of 36 had skin lesions on the head and half of them had external bleedings. All but one bird had subcutaneous haemorrhages. Cranial fractures were observed in 33 birds, with a mean score of 2.9 (range 1 to 4). Brain damage was found in the cerebrum and/or cerebellum, but two chickens showed no brain damage. All animals had submeningeal haemorrhages, with a mean score of 3.2 (range 1 to 5). Due to minimal brain damage in two chickens (no brain damage and/or low score of submeningeal haemorrhages), 34 out of 36 killings were considered accurate. According to the producers, killing by N2 was effective for 167 out of 173 chickens.

For CB, the producers were positive about safety, maintenance, low risk of fatigue, and ease of execution. Time- and cost-efficiency were scored lower. Furthermore, both producers also reported that the CB method was less successful, animal-friendly, executable in poultry houses, or the preferred method of the veterinarian. The producers reported two disadvantages of the CB method: the birds suffer from stress caused by the displacement of the animals out of the barn; it seemed difficult to regulate the pressure so that it is sufficient to kill the birds effectively without causing excessive external bleeding. The N2 method was scored positively for easiness of execution, success rate, and operator safety. The scores for animal-friendliness, time-efficiency, and risk of fatigue were intermediate. The producers gave a low score for feasibility to perform inside the stable, cost-efficiency, maintenance, and whether they considered it to be a method that would be preferred by the veterinarian. After testing, the producers mentioned the speed at which the birds were killed as an advantage, but the need to displace birds out of the barn, resulting in stress, as a disadvantage.

DISCUSSION

Besides manual CD, which is by far the most common method used for killing broiler chickens on commercial farms, alternative methods exist such as CB or N2. However, producers are rarely familiar with these alternative methods (Watteyn et al., 2020). Important criteria for a humane killing method include minimal distress for the animal and rapid and irreversible insensibility. In the first study, these parameters were measured for killing broilers by non-penetrating CB as compared to manual CD. N2 was not included in this study as these parameters were already investigated and reported by McKeegan et al. (2013). The benefits of replicating their study were considered inferior to the costs in terms of the additional number of birds that would need to be sacrificed for the trial. In addition, to achieve widespread use among poultry producers, any killing method ought to be safe, practical, and feasible in an on-farm context, and be perceived as such by the farmer or caretaker. This was investigated, albeit at a small scale, in the feasibility study with commercial broiler producers who were surveyed after they had been able to become acquainted with and test two alternative (and to them novel) killing methods (CB and N2). To our knowledge, this is the first study in which alternative methods are being tested and evaluated by farmers.

Effectiveness and Animal Welfare Study

A non-invasive indicator that was used in the first study was the latency to loss of a pain response, specifically pinching the comb. The absence of such a pain response indicates insensibility, as pain can only be experienced if the animal is conscious (EFSA, 2006; Erasmus et al., 2010a; Sandercock et al., 2014). For both CD and CB, the pain response was almost immediately absent.

TABLE 5 | Summary of the post-mortem examination after captive bolt (CB) in Effectiveness and Animal Welfare Study (= Experimental study) and Feasibility study, with the prevalence (in % and ratio) of injuries specific for CB, score (mean and range) of cranial fractures and submenigeal haemorrhages, and decision on the accuracy of execution of the killing method.

CB	Experimental Study (n=26)		Feasibility Study (n=36)	
	Prevalence (% – n/26)	Mean Score (range)	Prevalence (% – n/26)	Mean Score (range)
Skin lesions (head)	46.2 12/26		13.9 5/36	
External bleeding (eyes/nostrils/mouth)	88.5 23/26		50.0 18/36	
Subcutaneous haemorrhages (head/neck)	100.0 26/26		97.2 35/36	
Cranial fractures	96.2 25/26	3.6 (0-4)	91.7 33/36	2.9 (1-4)
Brain damage	96.2 25/26		91.7 33/36	
- *cerebrum*	*88.5 23/26*		*80.6 29/36*	
- *cerebellum*	*92.3 24/26*		*88.9 32/36*	
Submeningeal haemorrhages	96.2 25/26	4.3 (1-5)	100.0 36/36	3.2 (1-5)
- *cerebrum*	*92.3 24/26*		*72.2 26/36*	
- *cerebellum*	*96.2 25/26*		*94.4 34/36*	
Accurate execution	92.3 24/26		94.4 34/36	

The variation in time and the differences between ages can be explained by the measuring latency, i.e., the time between the application of the method and the first measurement of the reflex. Thus, chickens of all ages killed by CD as well as CB showed rapid insensibility. However, it should be noted that since the spinal cord is ruptured after CD, it is not clear whether a pain response is still possible. McKeegan et al. (2013) studied the brain function and behavioural responses of 5-week-old broilers following exposure to nitrogen in foam. They found a suppressed EEG (indicative of loss of consciousness) 18 s after submersion in foam, and a similar duration (15 s) was reported for the onset of wing flapping, and concluded that the onset of convulsions may indicate unconsciousness. Also, after blunt trauma, convulsions are a sign of successful mechanical stunning and indicate traumatic unconsciousness (EFSA, 2004; Cors et al., 2015). In the current study, the onset of convulsions was almost immediate for both CD and CB, which would suggest that birds lost consciousness within seconds. Erasmus et al. (2010c), however, claimed that the non-occurrence of convulsions could be related to an ineffective killing. Also, previous research stated that the onset of convulsions is not a useful indicator of brain function as these can be seen as involuntary neuromuscular spasms after brain anoxia induced by physical methods or nitrogen gassing (Hernandez et al., 2019; Martin et al., 2019). Furthermore, the pupillary light reflex can be used as an indicator of complete unconsciousness and even brain death (Croft, 1961; Erasmus et al., 2010c; McKeegan et al., 2013; Martin et al., 2016). We found a positive correlation between the loss of the pupillary light reflex and the cessation of convulsions and heartbeat. In contrast, Martin et al. (2019) could not correlate the loss of reflexes (nictitating membrane, pupillary light reflex, and rhythmic breathing) with an isoelectric signal on the EEG because the time to reflex loss was faster than the time to an isoelectric EEG. They warned that relying only on the reflexes to assume the bird is dead would be incorrect. In the current study, the pupil light reflex disappeared after approximately 1 min (or longer in 6-week-old birds) following manual CD, which is comparable to the duration reported by Martin et al. (2016; 2019). Baker-Cook et al. (2021) measured shorter intervals in loss of the pupillary light response (30 to 46 s). With CB, the loss of the pupil light reflex was much faster (approximately 10 s). This can be explained by the fact that this reflex is controlled by the midbrains and CB causes trauma specific to that location (Whittow, 2000). Baker-Cook et al. (2021) measured also shorter latency to loss of the pupillary light response after CB compared to CD in broilers at different ages. Additionally, Jacobs et al. (2021) found quick loss of reflexes (i.g. nictitating reflex) after CB in turkeys. Hence, these findings suggest that poultry killed by CB are almost immediately brain dead.

The moment birds stop having convulsions has also been associated with brain death (Dawson et al., 2009; Erasmus et al., 2010c). When relying on this indicator, brain death occurred much later (approximately 2-3 min for CD and 1-2 min for CB) than suggested by the pupillary light response. Interestingly, the cessation of convulsions coincided with cardiac arrest (R^2=0.62). This suggests that cessation of convulsions is related to clinical death, which is the cessation of heartbeat, following CD and CB. Bandara et al. (2019) also found a positive correlation between last convulsions and cessation of heartbeat after killing laying hens by CB. In this study, the end of convulsions was significantly faster after CB than after CD, suggesting that the chicks would die earlier after CB, but Baker-Cook et al. (2021) observed the opposite. Notwithstanding, killing by N2 in foam seems to be even faster, with an isoelectric EEG after 47 s, which is comparable with the time of cessation of movements (51 s) (McKeegan et al., 2013).

Post-mortem examination revealed that two CB killings were not performed properly. This seemed due to an inaccurate position of the barrel, which was placed too close to the frontal cortex. Other studies reported unsuccessful killing with CB (Erasmus et al., 2010a; Baker-Cook et al., 2021). Duration of convulsions and the heartbeat was prolonged in those two birds, while the killing was effective according to the non-invasive indicators (rapid absence of pain reflex and onset of convulsions). This emphasises the distinction between loss of consciousness and actual death. On the contrary, manual CD was executed accurately in all cases with lesions located between the skull and the first cervical vertebra. This indicates trauma to the medulla oblongata, especially due to the traction when CD was performed. Consequently, this should result in a lack of pain reflex, which was immediately absent. Damage to the medulla also increases heart rate (Orosz and Antinoff, 2016). As the heartbeat of a broiler is very fast and difficult to count manually with a stethoscope, no information on the exact heart rate is available. The traction also causes a rupture of the blood vessels in the neck region, resulting in cerebral ischemia and brain death (Shi and Pryor, 2002; Erasmus et al., 2010a). In this study, all birds killed by CD had large haemorrhages at the neck region, indicating that the blood supply to the brain was interrupted. When using mechanical CD methods without traction (such as Koechner Euthanizing device, burdizzo, neck crusher), the likelihood of damage to the blood vessels is reduced, resulting in a slower onset of unconsciousness (Gregory and Wotton, 1990; Erasmus et al., 2010a; Jacobs et al., 2019; Baker-Cook et al., 2021; Watteyn et al., unpublished data). A good performance of CD with damage to the medulla oblongata and blood vessels emphasises the importance of good training. Especially for younger birds, it is not always easy to dislocate close to the skull (Baker-Cook et al., 2021).

Beside the speed and duration of loss of consciousness and death, the amount of stress inherent to a killing method (including the handling and restraining that is required) determines the animal welfare impact. For example, holding birds upside down may cause distress and fear due to the compression of the heart and lungs by the viscera (Broom et al., 1986; EFSA, 2019). Inversion may occur when birds are carried by the operator to the place of killing, but also for example during the process of restraint in a cone. Stress indicators were not measured in this study, although conclusions can be drawn from other studies. Killing by CD is done immediately and usually on the spot, without carrying the birds upside down. Accordingly, stress due to inversion is not likely. CB killing causes a loud noise, so it is advisable to carry it out outside the barn in order not to frighten the other birds. In addition, it is advisable to restrain the birds in a cone (or something similar in which the birds are restrained and head and neck are exposed), which might cause additional stress to the birds being killed due to the inverted position (EFSA, 2019). Since killing with N2 also must be done outside the barn, as the unit is difficult to move and requires an electrical connection, the selected birds may also be stressed by carrying them (EFSA, 2019). Once inside the container, where the birds sit upright, aversion to the foam is low and the EEG pattern and physiological responses were very rapid (McKeegan et al., 2013), indicating low stress levels.

Small Scale Feasibility Study

Watteyn et al. (2020) revealed that producers' knowledge of, and experience with, killing methods other than CD are very limited. Many respondents indicated a willingness to learn more about these techniques though. Therefore, this was the focus of this study, albeit on a small scale. But the results give an interesting view on the opinion of producers and the effect of hands-on experience. The alternative N2 method is very promising, as the high expansive foam used allows for immediate immersion in nitrogen compared to conventional gas methods, which rely on circulating the container with the gas until the desired concentration is reached (McKeegan et al., 2007; Gerritzen and Sparrey, 2008).

In the small-scale study, inaccurate killings were observed in the post-mortem examinations after CB. Moreover, the mean scores of cranial fractures and haemorrhages were lower when performed by producers compared to researchers, which indicates that the impact of CB was lower and could result in an ineffective killing. This highlights the importance of effective training. Not all accurately killed birds with CB had macroscopic brain damage. However, extended submeningeal haemorrhages (score higher than 1) cause high pressure on the brain and a reduced blood flow to the brain, resulting in dysfunction followed by insensibility and death (Erasmus et al., 2010c). The occurrence of external bleeding in half of the chickens can be considered a disadvantage because of the additional biosecurity hazard (Jacobs et al., 2021). After post-mortem observations of N2-killed birds, McKeegan et al. (2013) found no obstructions of the airway by foam. This evaluation was not performed in the current study, as the presence of foam is only temporary and would not be seen due to the time lag of transporting the dead birds from the farm to the pathology room. However, the method seems to be highly effective as 96.5% of the birds were successfully killed by the producers. The reason for the unsuccessful killing of the six birds was an empty bottle of N2, which could be avoided as a screen on the bottle indicates the remaining percentage of N2. The manual describes a minimum of 10% prior to starting a cycle. Although the CD method was not included in this study, it would be interesting to further investigate the implementation of this method among broiler producers.

The producers were in general more positive about N2 compared to CB. The N2 method was considered easy to execute, with a high success rate and a good time efficiency. The main disadvantages for both devices were the high cost of the equipment (€ 1200 and € 3200 for CB and N2, respectively) and not being able to use them in the barn. The producers did not think of CB as animal-friendly. They found it quite time-consuming, which increased stress for the birds, and sometimes had to discharge the CB twice. Although our results showed a very quick death after CB, it requires training to perform it fast and correctly. They indicated that the CB method would be more suitable for large poultry (e.g., turkeys). During the daily control in the barn, broiler producers might select strictly, resulting in a high number of chickens that must be killed. If so, N2 could be useful as multiple chickens can be placed in the container, while killing by CB requires killing birds individually.

CONCLUSION

In conclusion, killing by manual CD as well as CB causes rapid and irreversible insensibility, both methods can be considered humane, independent of the age of the broilers. Brain death will occur later after CD compared to CB. However, for the CB method, there is more handling stress and thorough training of the operator is essential to obtain high effectiveness. Although the producers considered N2 as a better killing alternative than CB, both methods are expensive and will probably not replace manual CD. In the future, extensive information, proper training, and eventually financial support will have to be provided to make an investment in these alternative methods possible and to become routine killing methods.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by ethical committees of Flanders Research Institute for Agriculture, Fisheries and Food (2017/296), Ghent University (2018/10), and by the Department of Environment – Animal Welfare of the Flemish Government. Written informed consent was obtained from the owners for the participation of their animals in this study.

AUTHOR CONTRIBUTIONS

AW, AG, BA, LJ, CM, and FT designed the research. AW and AG carried out the experimental work. AW and BA analysed and interpreted the data. AW wrote the first draft of the paper. All authors reviewed and approved the final paper.

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