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Head to cloaca and head-only stunning of broilers

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Abstract
The alternative stunning methods head cloaca and head only are examined for inducing unconsciousness and affect on meat quality. When both methods are applied they induce unconsciousness without compromising the meat quality. It is recommended to develop the equipment for practical application and to implement the methods in commercial slaughterhouses.

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Dit onderzoek is uitgevoerd in opdracht van het Ministerie van Landbouw, Natuurbeheer en voedselkwaliteit.

Voorwoord

In de 80-er jaren van de vorige eeuw is in binnen- en buitenland veel onderzoek verricht naar het effectief verdoven van pluimvee. De resultaten waren destijds helaas niet eenduidig. Toch is algemeen geaccepteerd en in regelgeving vastgelegd dat vleeskuikens die worden verdoofd in een waterbad, minimaal 100 mA per dier toegediend moeten krijgen. Deze minimum stroomsterkte wordt door verschillende onderzoekers te laag geacht. Algemeen wordt nu aangenomen dat bij de voorgeschreven stroomsterkte van 100 mA in combinatie met een frequentie van 50 Hz nog 10% van de vleeskuikens niet effectief wordt verdoofd. Een ineffectieve verdoving is niet altijd zichtbaar omdat deze het dier meestal wel fysiek immobiliseert, maar er niet voor zorgt dat het dier zodanig buiten bewustzijn is dat pijn, stress en ongemak niet gevoeld worden. Wanneer bij 100 mA hogere frequenties gebruikt worden dan 50 Hz zal het percentage kuikens dat onvoldoende verdoofd is aanzienlijk hoger liggen dan 10 %. Bij hogere frequenties wordt door de EFSA tevens een hogere stroomsterkte geadviseerd. Een nadeel van een hogere stroomsterkte is dat het vleeskuiken meer kans heeft te verkrampen. Dit veroorzaakt bloedingen en botbreuken in het karkas. Daarom wordt naar alternatieven voor deze werkwijze gezocht. De druk op het toepassen van alternatieven is toegenomen na uitbrengen van het rapport over het elektrisch verdoven van pluimvee (Hindle et al., 2010), dat duidelijk maakt dat het klassieke elektrische waterbad als verdovingssysteem in te veel gevallen niet volstaat. In het voorstel voor een nieuwe EU VERORDENING Nr. 1099/2009 VAN DE RAAD van 24 september 2009 inzake de bescherming van dieren bij het doden worden hogere stroomsterkten bij hogere frequenties verplicht gesteld.

Als alternatief voor de conventionele waterbadmethode is een kop-kop methode ontwikkeld, waarbij de stroom enkel door de kop gaat in plaats van door het gehele lichaam inclusief het hart. Oorspronkelijk is de verdover ontwikkeld met droge elektroden en deze uitvoering is in dit onderzoek getest. Momenteel is als aanvulling hierop een kop-kop verdover met bevochtiging in ontwikkeling, maar resultaten hiervan zijn nog niet beschikbaar.
Een andere ontwikkeling is de kop-cloaca methode. Door stroom toe te dienen via de cloaca (in plaats van via de poten) naar de kop wordt de elektrische weerstand van het dier sterk gereduceerd. De kop-cloaca methode is alleen onder laboratoriumomstandigheden onderzocht. Er is nog een ontwikkeltraject nodig voor het in de praktijk kan worden toegepast.

Het onderzoek is uitgevoerd in opdracht van het ministerie van LNV en vond plaats in een commercieel slachthuis, waar een ruimte was gecreëerd voor het plaatsen van de verdovingsmachines. De vleeskuikens werden ter beschikking gesteld door de directie van het slachthuis. De kop-kop en kop-cloaca verdovers zijn ontwikkeld en geleverd door respectievelijk Tiel Engineering BV en Meijn Food Processing Technology BV. Beide bedrijven leverden ook de noodzakelijke technische ondersteuning.

This research was performed for and sponsored by the Dutch Ministry for Agriculture, Nature and Food Quality.

Preface

In broiler chicken processing, it is common for the birds to be hung at shackles (positive electrode) with their legs, and electrocution is done electrically by contact of the head with an electrified water bath (negative electrode). Insufficient currents may physically immobilize the bird but may not prevent the perception of pain, stress, or discomfort by the bird. A minimum current of 100 mA per bird is required to stun-kill the bird (Council Directive, 1993; Council Regulation, 2009). This required minimum current for broilers in the European Union increases quality defects (e.g., hemorrhages, broken bones) in carcasses and broiler meat. It is apparent that there can be a conflict between bird welfare and carcass quality when using electrocution by the conventional water bath method as stun-kill procedure. Therefore, alternative methods for stunning broilers must be explored.

Samenvatting

In de wetgeving is vastgelegd dat slachtdieren voorafgaand aan het doden door verbloeden op een adequate manier moeten worden verdoofd. Met een adequate verdoving wordt een directe staat van bewusteloosheid en ongevoeligheid bereikt die aanhoudt tot de dood intreedt door verbloeden.
De meest toegepaste methode voor het verdoven van pluimvee is het gebruik van een elektrisch waterbad. Bij binnenkomst in de slachterij wordt het dier uit de transportkrat gehaald en met de poten in de slachthaak aan de slachtlijn gehangen. Vervolgens wordt het dier met de kop door een waterbad gevoerd. Door contact te maken met het water gaat er een elektrische stroom door het dier lopen van het waterbad naar de slachthaak.
Een commercieel nadeel van de conventionele elektrische waterbadverdoving is dat bij stroomsterkten die nodig zijn voor een adequate bedwelming van alle dieren, bij een aanzienlijk aantal dieren zorgt voor bloedingen in borst- en pootspieren en gebroken botten in het karkas. Voor kippen geldt dat de kans op dergelijke bloedingen en botbreuken toeneemt als de gemiddelde stroomsterkte hoger ligt dan 105 mA bij gebruik van een conventionele laagfrequentieverdover (50 Hz AC). Deze bloedingen zijn zichtbaar als roodbruine stipjes in het vlees en worden uit esthetisch oogpunt onwenselijk geacht. Om deze reden wordt er naar alternatieve verdovingsmethoden gezocht. Alternatieven met perspectief zijn de kop-cloaca en kop-kop verdovingsmethoden.

Kop-cloaca verdoven

Het doel van deze pilotproeven is een indicatie te verkrijgen over een correcte verdoving en verbloeding bij de kop-cloaca verdovingsmethode. De deel aspecten zijn:

- de wijze van fixatie tijdens het verdoven,
- een verdoving van voldoende lange duur van het dier (voltage, stroomsterkte, frequentie, pulsen),
- een verbloeding, die tot een snelle dood leidt,
- een minimale kans op beschadiging van het karkas.

Methode

Voor het bepalen van een correcte manier van verdoven zijn 55 vleeskuikens random geselecteerd uit koppels die aan de slachterij zijn geleverd. Het verdoven gebeurde met 1 dier tegelijk. Het kuiken werd met de poten in de slachthaak gehangen, waarna het individuele waterbakje omhoog kwam en de cloacapin omlaag zakte zodat deze op de cloaca rustte op het moment dat de kop het water raakte. Op dat moment ging gedurende 1 tot 1,5 s een stroom lopen van respectievelijk 70 mA i.c.m. 70 Hz of 100 mA i.c.m. 100 Hz (AC blokvorm). Na het verdoven zijn op vastgestelde tijdstippen pijnstimuli toegediend waarop het dier al dan niet (zichtbaar op het EEG) reageerde. Op de EEG en ECG zijn voor, tijdens en na het verdoven de hersen- en hartactiviteit geregistreerd; deze zijn op een later tijdstip geanalyseerd.
Voor de vleeskwaliteitsmeting is een groep van 60 vleeskuikens geselecteerd uit een koppel geleverd aan de slachterij. De helft van de kuikens is met de kop-cloaca methode verdoofd en de andere helft met de conventionele waterbadmethode. Na het slachten zijn de temperatuur, zuurgraad, kleur, kookverlies en scheurweerstand van de filets gemeten. Bloedingen in de spieren van de filets en poten zijn visueel beoordeeld.

Resultaten

Op de EEG's is een algemeen epileptiform insult waargenomen bij vleeskuikens die verdoofd zijn gedurende 1 en 1,5 s met 70 mA – 70 Hz, en bij vleeskuikens die verdoofd zijn gedurende 1,5 s met 100 mA – 100 Hz (AC blokvorm). Het epileptiform insult omvat een tonische-, klonische- en uitputtingsfase, gevolgd door pieken en alpha, beta, theta en delta golven. De kuikens verdoofd met 70 mA zijn volgens de cordimanus scoreanalyse ongeveer 15 s of langer verdoofd en de kuikens verdoofd met 100 mA zijn volgens deze analyse 20 s of langer verdoofd. Binnen een betrouwbaarheidsinterval van 95% en met inachtneming van het aantal dieren met een betrouwbaar EEG (n=28 bij 70 mA; n=27 bij 100 mA) ligt de kans op een effectieve verdoving van alle vleeskuikens tussen 0.9 en 1.0 bij toediening van 70 mA – 70 Hz – 1,5 s evenals bij toediening van 100 mA – 100 Hz – 1,5 s (AC blokvorm).
Na slachten en koelen is the pH significant ($p < 0.05$) lager in de groep dieren verdoofd volgens de kop-cloaca methode in vergelijking met de groep dieren verdoofd volgens de conventionele

waterbadmethode. Ook is de scheurweerstand hoger, wat duidt op malser vlees. Bij kop-cloaca verdoofde kuikens is het percentage filets en poten zonder bloedingen hoger en het percentage ernstige bloedingen lager dan die verdoofd in het waterbad.

Conclusies

Vleeskuikens kunnen effectief worden verdoofd met de kop-cloaca methode door toediening van een gecontroleerde stroom van 70 mA i.c.m. 70 Hz en 100 mA i.c.m. 100 Hz (AC blokvorm) voor de minimale duur van 1 of 1,5 s. Het vlees van de filets is mogelijk iets malser en er zijn minder bloedingen geconstateerd in de karkassen van de dieren verdoofd volgens de kop-cloaca methode vergeleken met de dieren verdoofd volgens de conventionele waterbadmethode.

Aanbevelingen

Aangezien de periode van bewusteloosheid het langst is bij 100 mA – 100 Hz – 1,5 s wordt aanbevolen deze procedure te volgen en binnen 10 s na verdoven van het dier, alle belangrijke bloedvaten in de hals aan te snijden. Zo wordt voorkomen dat het kuiken weer bij bewustzijn komt tijdens het verbloeden. De kop-cloaca verdover kan verder worden ontwikkeld om deze praktijkrijp te maken en het apparaat zo spoedig mogelijk in commerciële slachthuizen te implementeren. Het welzijn en de vleeskwaliteit kunnen worden verbeterd bij een goed functioneren van de apparatuur. De kwaliteit van het welzijn van het kuiken en ven het vlees in het karkas kunnen in een onderzoek worden geëvalueerd.

Kop-kop verdoven

Het doel van deze pilotproeven is een indicatie te verkrijgen over een correcte verdoving en verbloeding bij de kop-kop verdovingsmethode. De deel aspecten zijn:

- de wijze van fixatie tijdens het verdoven,
- een verdoving van voldoende lange duur van het dier (voltage, stroomsterkte, frequentie, pulsen),
- een verbloeding, die tot een snelle dood leidt,
- een minimale kans op beschadiging van het karkas.

Methode

Voor het bepalen van een correcte manier van verdoven zijn 47 vleeskuikens random geselecteerd uit koppels die aan de slachterij zijn geleverd. Het verdoven gebeurde met 1 dier tegelijk. Het kuiken werd met de poten in de slachthaak gehangen, waarna de kop in de goede houding werd gebracht door het liften van de conus. Vervolgens fixeerden 2 elektroden de kop en ging er door de kop een stroom lopen met een ingestelde waarde van 240 mA en 50 Hz (AC sinusvorm) met een duur van 0.5, 3 of 5 s. Na het verdoven zijn op vastgestelde tijdstippen pijnstimuli toegediend waarop het dier al dan niet (zichtbaar op het EEG) reageerde. Op de EEG en ECG zijn voor, tijdens en na het verdoven de hersen- en hartactiviteit geregistreerd; deze zijn op een later tijdstip geanalyseerd.
Voor de vleeskwaliteitsmeting is een groep van 50 vleeskuikens geselecteerd uit een koppel geleverd aan de slachterij. De helft van de kuikens is verdoofd met de kop-kop methode en de andere helft is verdoofd met de conventionele waterbadmethode. Na het slachten zijn de temperatuur, zuurgraad, kleur, kookverlies en scheurweerstand van de filets gemeten. Bloedingen in de spieren van de filets en poten zijn visueel beoordeeld.

Resultaten

Op het EEG is een algemeen epileptiform insult met opeenvolgend een tonische-, klonische-, uitputtings- en herstelfase waargenomen na de stroomtoediening gedurende 0,5, 3 of 5 s. Deze kuikens zijn volgens een visuele analyse respectievelijk 30, 44 en 65 s en volgens de cordimanus scoreanalyse 18, 12 en 16 s bewusteloos. Binnen een betrouwbaarheidsinterval van 95% en met in achtneming van het aantal dieren met een betrouwbaar EEG (n=47) is de kans op een effectieve verdoving van alle vleeskuikens tussen 0.95 en 1.00 met een stroom van gemiddeld 190 ± 30 mA (AC sinusvorm) gedurende 0,5 s.
Na slachten en koelen is the pH significant ($p < 0.05$) lager in de groep dieren verdoofd volgens de kop-kop methode in vergelijking met de groep dieren verdoofd volgens de conventionele waterbadmethode. Bij kop-kop verdoofde kuikens is het percentage filets zonder bloedingen 80% en

er zijn bovendien geen filets met ernstige bloedingen. Ook zitten er minder bloedingen in de poten dan bij die verdoofd in het waterbad.

Conclusies

Vleeskuikens kunnen effectief worden verdoofd met de kop-kop methode met een elektrische stroom van 190 ± 30 mA i.c.m. 50 Hz (AC sinusvorm) met een duur van 0,5 s. De karkassen van vleeskuikens verdoofd volgens de kop-kop methode vertoonden veel minder bloedingen, waardoor de kwaliteit van het vlees wordt verhoogd vergeleken met karkassen van kuikens verdoofd met het waterbad.

Aanbeveling

Aangezien de weerstand bij individuele dieren sterk kan verschillen, wordt bij implementatie in de praktijk aangeraden om de stroom in the stellen op 250 mA (gemiddelde + 2*SD) en om bijkomen te voorkomen binnen 10 s alle belangrijke bloedvaten aan te snijden. Het wordt aanbevolen om de kop-kop verdover verder te ontwikkelen en deze praktijkrijp te maken zodat het apparaat zo spoedig mogelijk in commerciële slachthuizen kan worden geïmplementeerd. Het welzijn en de vleeskwaliteit kunnen worden verbeterd bij een goed functioneren van de apparatuur. De kwaliteit van het welzijn van het kuiken en ven het vlees in het karkas kunnen in een onderzoek worden geëvalueerd.

Summary

Introduction

Current legislation demands that all birds are immediately rendered unconscious at stunning and that they remain insensible until death ensures. Use of the water bath is a legal electrical stunning method for poultry. In order for a stun to conform with the demands of legislature several aspects of the water bath method are of importance to its successful execution. The legal minimal current for an individual bird in the water bath is 100 mA. Current Dutch and EU legislation on water bath stunning is incomplete and should include details concerning wave form and frequency alongside current levels in recognition of the large impedance variation between individual birds. The recommended minimum current for broilers in the EU increases quality defects (haemorrhages, broken bones) of carcasses and broiler meat. It is apparent that there can be a conflict between animal welfare and carcass quality using electrocution as stun-kill procedure. Therefore, the challenge still remains of providing an alternative stunning method with an effective threshold current that will induce consciousness and insensibility in broilers without compromising carcass quality.

Head-cloaca controlled current stunning

The objective of the study was to identify an electrical current and exposure duration to render broiler chickens unconscious instantaneously at slaughter using a head to cloaca water bath stunner. The stunning methods were assessed using EEG and ECG. Meat quality after head-cloaca electrical stunning was compared with the currently applied water bath method in a commercial setting. Analysis of pH, colour, cooking loss and shear force from fillets during storage and scoring of blood splashes were chosen as parameters for product quality.

Method

To assess a correct way to stun 55 broilers were used and obtained as they were delivered at the slaughterhouse from a commercial farm. Stunning was done with one bird at the time. The birds were hung at shackles with their legs, after which the water bath was lifted and the cloaca pin lowered so that the pin touched the cloaca when the head reached the water. At that moment current started to flow from the water bath via the animal to the hook for 1 or 1.5 s s using a set current of 70 mA combined with 70 Hz or 100 mA combined with 100 Hz (AC block shape). The EEG and ECG were recorded from 30 s before and 2 minutes after stunning. The response of each animal to a pain stimulus (comb pinching) was observed for 2 minutes following the stun in order to assess unconsciousness.
To assess meat quality a group of 60 broilers were selected from a group that was delivered to the slaughterhouse. Half of the group was stunned with the head-cloaca method and the other half was stunned as control group with the conventional water bath method available at the slaughterhouse. Temperature, pH, colour, cooking loss and shear force in breast muscle (*P. major*) were measured post mortem.
Hemorrhages in breast (dorsal side of *P. major* and *minor*) and left and right thigh muscles (medial side) were quantified by a visual grading system.

Results

On the EEG recordings a general epileptiform insult was observed when applying a current of 100 mA combined with 100 Hz for a duration of 1.5 s. This general epileptiform insult shows a tonic, clonic and exhaustion phase, followed by a mix of spikes, alpha, beta, theta and delta waves. These birds may have been unconscious for approximately 20 s or more, according the cordimanes score analyses. Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=27), the chance on an effective stun of all broilers lies between 0.9 and 1.0 with a current of 100 mA – 100 Hz – 1,5 s (AC block shape). The heart rate decreased significantly ($p < 0.05$) after stunning and recovered afterwards.
A general epileptiform insult showing the same signals was also observed on the EEG of birds stunned with 70 mA combined with 70 Hz for a duration of 1,5 s. According to the cordimanes score analyses, these birds may have been unconscious for approximately 15 s or more. Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=28), the chance on an effective stun of all broilers lies between 0.9 and 1.0 with a current of 70 mA – 100 Hz –

1,5 s (AC block shape). The heart rate decreased significantly ($p < 0.05$) after stunning and recovered afterwards.
The pH after chilling is significantly ($p < 0.05$) higher in the head-cloaca stunned group and the shear force lower ($p < 0.01$) compared to the conventional water bath stunned group, which means the meat is more tender. The percentage of fillets and legs without blood splashes is higher and the percentage with severe blood splashes lower to none.

Conclusions

It can be concluded from this experiment that broilers are effectively stunned with the head-cloaca method by using a controlled current of 70 mA combined with 70 Hz or 100 mA combined with 100 Hz (AC block shape) for the duration of minimal 1 s. The meat in the fillet might be more tender and there are less blood splashes in both fillet and legs of bird stunned head-cloaca compared to birds stunned in a conventional water bath.

Recommendations

Because the period of stunning is longest at 100 mA – 100 Hz – 1.5 s, it is recommended to use this procedure followed by a neck cut within 10 s. This prevents the bird from recovering to consciousness during exsanguination.It is recommended to develop the head-cloaca equipment further for practical implementation in broiler slaughter houses. Unconsciousness and insensibility can be induced individually with and this method reduces the amount of product devaluation,

Head only electrical stunning

The objective of the study was to evaluate behavioural, neural and physiological responses of broilers after: head only electrical stunning restrained by their feed and by their body in a cone. Analysis of pH, colour, cooking losses and shear force from fillets during storage and scoring of blood splashes were chosen as parameters for evaluation of product quality.

Method

To asses a correct way to stun 47 broilers were used and obtained as they were delivered at the slaughterhouse from a commercial farm. Stunning was done one bird at the time.

The birds were hung at shackles with their legs and stunning is done electrically via two pointed electrodes on both sides of the head. Then the current ran through the head for 0.5 to 5 s. s using a set current of 240 mA combined with 50 Hz (AC sinusoidal shape). The EEG and ECG were recorded from 30 s before and 2 minutes after stunning. The response of each animal to a pain stimulus (comb pinching) was observed for 2 minutes following the stun in order to assess unconsciousness.
To asses meat quality a group of 50 broilers were selected from a group that was delivered to the slaughterhouse. Half of the group was stunned with the head only method and the other half was stunned as control group with the conventional water bath method available at the slaughterhouse. Temperature, pH, colour, cooking loss and shear force in breast muscle (*P. major*) were measured post mortem.
Hemorrhages in breast (dorsal side of *P. major* and *minor*) and left and right thigh muscles (medial side) were quantified by a visual grading system

Results

On the EEG recordings a general epileptiform insult was observed when applying a voltage the current for a duration of 0.5, 3 or 5 s. This general epileptiform insult shows a tonic phase, followed by a clonic phase and an exhaustion phase, after which the birds recovered. These birds may have been unconscious for approximately 30, 44 and 65 s, using a visual analysis. According the cordimanes score analyses these durations were 18, 12 and 16 s, respectively. Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=47), the chance on an effective stun of all broilers lies between 0.95 and 1.00 with an average current of 190 ± 30 mA – 50 Hz (AC sinusoidal shape) is used.

After stunning the ECG revealed fibrillation. The heart rate decreased significantly ($p < 0.05$) after stunning and recovered afterwards
The pH after chilling is significantly ($p < 0.05$) lower in the group stunned head only compared to the water bath group. The percentage of fillets free of blood splashes was 80% with no severe blood splashes in the carcasses stunned head only. This was 16% and 12% respectively compared to those stunned in the commercial water bath. Such high differences were not observed in the legs.

Conclusions

It can be concluded that, broilers may be insensible and unconscious after head-only electrical stunning with pinned electrodes using an average current of 190 ± 30 mA (sinusoidal AC) for 0.5 s.

Recommendation

For practical implementation a set current of 250 mA (average + 2*SD) is recommended to overcome individual differences in resistance. To prevent recovery the stun should be followed by a neck cut within 10 s by severing all the major blood vessels. Since carcass quality is limited compromised such equipment should be further developed for practical application and commercial use.

Hierbij de tekst uit EFSA rapport 2004:

The minimum root mean square (RMS) currents of sine wave AC necessary to stun chickens and turkeys effectively were found to be 240 and 400 mA, respectively, whilst using conventional stunning electrodes made of three pins (Gregory and Wotton, 1990a and 1991b). These studies, however, involved prolonged administration of currents (minimum of 5 sec) using a constant voltage stunner. Nevertheless, when neck cutting was performed by severing all the major blood vessels in the neck within 10 to 15 sec from the end of stun, it prevented the return of consciousness in these birds. This may also apply to the use of a variable voltage / constant current stunner because, although the source of current would affect the rate of induction of stun, it is unlikely to alter the duration of unconsciousness and insensibility in adequately stunned poultry. However, **there is no published scientific literature concerning this.**

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1 Introduction

According to the EU Council Directive (1993) on the protection of animals at the time of slaughter it is stated that horses, ruminants, pigs, rabbits and poultry brought into abattoirs for slaughter shall be a) moved and if necessary temporarily housed, b) restrained and c) stunned before slaughter. Animals must be restrained in an appropriate manner, so as to spare them any avoidable pain, suffering, agitation, injury or contusions. Animals must not be suspended before stunning or killing. However, poultry and rabbits may be suspended for slaughter provided that appropriate measures are taken to ensure that they are in a sufficiently relaxed state for stunning. Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to special gas mixtures.
A specially applied method of electro-anaesthesia is widely used for the stunning of slaughter animals. Electrical stunning is based on the induction of a general epileptiform insult ('grand mal' or seizure-like state) by passage of an electrical current through the head and brain. Provided that sufficient current is administered through the head of an animal a general epileptiform insult (spreading across parts of the brain stimulating many cells) will occur. The epileptic process is characterized by rapid and extreme depolarization of the membrane potential and development of a synchronized electrical response. This can be measured and observed on the recorded electroencephalogram (EEG) as such an insult produces relatively small waves increasing in amplitude in the tonic phase (rigid), and decreasing in frequency in the clonic phase (high motor activity in muscles) resulting ultimately in a period of strong depression of electrical activity in pigs, sheep, calves, poultry and fishes (Lambooij 2004) However, the general epileptiform insult seen on the EEG of some birds and fishes is characterized by a tonic/clonic phase and a quiescent phase. The duration of the insults differs between species. Since human subjects are known to loose consciousness during the three phases of a general epileptiform insult, by analogy, other mammals are also assumed to be unconscious and insensible (Lambooij 2004). .
The insight into the stunning process that has come from neuro-physiological studies is of important significance. Assessment of more parameters than general epileptiform insult and analgesia may support the humaneness of the stunning and killing system. EEG and neurotransmitter release measurements have been used to assess the effects of electrical head-only stun duration on welfare (Cook,1999,1992,1995,1996; Lambooij, 2004). An understanding of the physiological mechanisms underlying the effects of electrical stunning may help to clarify the effect of several conditions on the effectiveness of stunning and killing. Stress before killing increases some neurotransmitters, which may affect the post stun reflexes and state of unconsciousness (Cook, 1999). Combining head-only stunning with exsanguination has a synergistic affect on the release of glutamate and aspartate, which increases the duration of unconsciousness (Cook, 1996). Sticking following a stun should be carried out as promptly as possible when using head-only stunning as it takes time depending on the species before brain responsiveness is lost following sticking (Anil, 1999; Hoenderken, 1978).). It is widely recognized that inducing a cardiac arrest at stunning has distinct welfare advantages: 1) it results in a rapid loss of brain function, 2) it ensures that the animal will not regain consciousness and 3) it does not depend on the operator performing an accurate stick (Anil, 1991; Gregory, 1994; Wotton et al., 1992).
Various stunning methods and electrical parameters have been reported to have a different effect on post-mortem rigor development (Devine et al., 1984; Gregory, 1984; Hillebrand et al., 1996; Bilgili, 1999, 1992; Roth et al., 2002, 2003). The post mortem metabolism is largely a consequence of indirect stimulation through nervous pathways. Broken vertebrae can occur when stunned with head-to-back electrode positioning if the voltage and current are too high (Wotton et al., 1992; Troeger and Woltersdorf, 1990). Sinusoidal alternating currents of 50 Hz have a large stimulatory effect on skeletal muscles which can be reduced by increasing the current frequency to an extent that prevents the occurrence of broken backs (Gregory et al., 1995) The prevalence of broken vertebrae and pelvises could be reduced to zero by increasing the frequency from 50 to 1500 Hz. The drawback of this approach is that the fibrillating effect on the heart is also reduced (Anil and McKinstry, 1991;Wotton et al., 1992) Haemorraghes can be induced by stunning and killing, however, the underlying mechanism is considered to be multi-factorial (Kranen et al., 2000; Troeger and Woltersdorf, 1991. Gregory et al., 1995). Investigations revealed that the morphology of haemorrhages was dependent on the tissue in which they occurred. In the pectoral muscles extravasating blood was found to follow the direction of the muscle fibres. In fat tissue, the majority of haemorrhages had a petechial appearance. More diffuse haemorrhages were found in loose connective tissue ((Kranen et al., 2000; Hillebrand et al., 1996). The histological study of haemorrhages in different types of muscles showed that the morphological appearance of the blood extravasation is determined by the structure of the tissue as well as by the amount of blood leaving the circulation.

2 Head-cloaca controlled current stunning

2.1 Introduction

Electrical stunning is normally used to induce unconsciousness during cutting and bleeding for reasons of animal welfare in the EU or to induce immobilization to facilitate automatic neck cutting in the U.S. In a water bath, in which electrical current is applied to the whole body, a minimum current of 120 mA per bird is recommended in the EU to induce unconsciousness and cardiac arrest (Gregory and Wotton, 1990). This recommended minimum current for broilers in the EU increases quality defects (haemorrhages, broken bones) of carcasses and broiler meat (Veerkamp and de Vries, 1983; Gregory and Wilkins, 1989). It is apparent that there is a conflict between welfare and meat quality under the electrical water bath stunning and killing procedure. Therefore, alternative methods for stunning broilers have to be explored.
Essential to the success of electrical stunning of poultry are certain aspects including adequate contact between bird and electrodes, level of current administered, duration of the stun and reduction in impedance. A study was designed in continuation to an earlier pilot study performed using a specially designed penetrative or non-penetrative electrode (Lambooij et al., 2008b). This study was envisaged to distinguish alternative methods of improving the contact between broiler and electrodes. It was hypothesized that the placement of electrodes in order to bypass the feet and legs would result in efficiency gains by utilizing lower currents. Additionally, use of alternatives waveforms to the standard sinus waveform could also improve efficient use of electricity. During this study the broilers were restrained in a conventional shackle framework and individual stuns were performed using a non invasive double or single electrode placed on or in the proximity of the cloaca. (Lambooij et al., 2008 a,b). The effects on consciousness of combinations of current, voltage and frequency settings were evaluated using electroencephalography (EEG) and electrocardiography (ECG).
To determine the effect of the electrical current on brain and heart their activity can be measured by recording the electrical potentials. Recording of an EEG (electro-encephalogram) is necessary to determine whether an electric current has been sufficient to induce a general epileptiform insult (grand mal seizure) indicating unconsciousness (Wageneder & Schuy, 1967). During high current electrical stunning, the heart is affected, which is recordable on an ECG (electro-cardiogram).
The objective of the study was to identify an electrical current and exposure duration to render broiler chickens unconscious instantaneously at slaughter using a head to cloaca water bath stunner. The stunning methods were assessed using EEG and ECG. Meat quality after head-cloaca electrical stunning was compared with the currently applied water bath method in a commercial setting. Analysis of pH, colour, cooking losses and shear force of fillets during storage and scoring of blood splashes were chosen as parameters for product quality evaluation.

2.2 Materials and methods.

2.2.1 Animals.

Fifty-five broilers delivered at the slaughterhouse from a commercial farm were used. Before transport the animals had a feed withdrawal period of 6 h and were transported in crates. During the experiment the birds were placed one by one in a specially developed head to cloaca restrainer using a controlled current stunner. (Figure 1) (Meijn Food Processing Technology BV, Oostzaan, Netherlands). The birds were hung individually by the feet from shackles and electrically stunned by submersion of the head of the bird under water. The water was one electrode and a dry steel U shaped electrode placed at or around the cloaca was the opposite electrode. After shackling the cloaca electrode was moved downwards and placed in position. Then the water bath was lifted and the bird immediately stunned for 1 or 1.5 s.
After the stunning experiment the broilers were weighed and breast and leg muscles were visually controlled for blood splashes (yes or no).

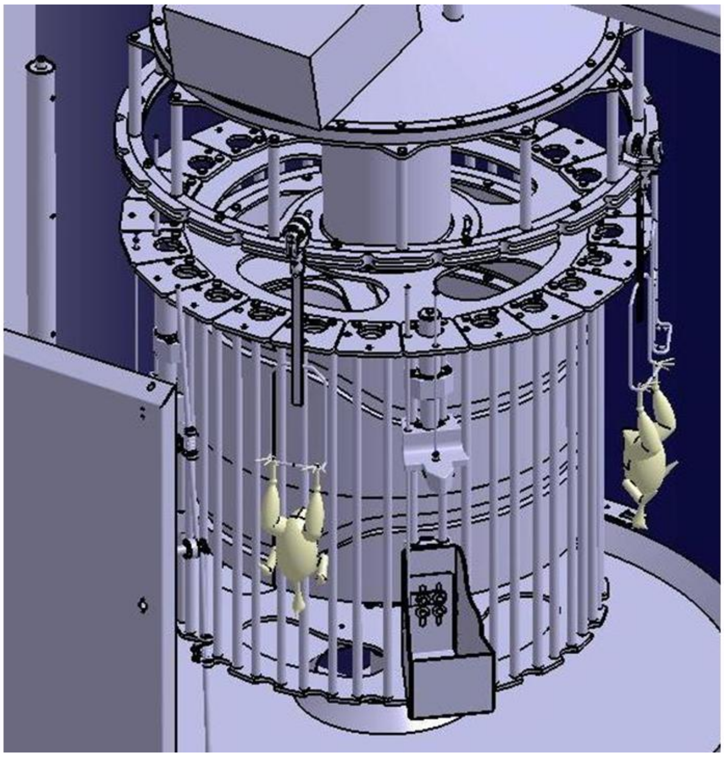

Figure 1: A developed carousel for stunning of individual broilers head to cloaca with a controlled current.

2.2.2 Experimental design

Each broiler was hung individually by the feet from the shackles and stunning was performed as the birds' head was immersed into a bath. The electrical current ran from head to rear of the bird. At the rear a single bar was lowered into position in close proximity to the cloaca. This stainless steel bar formed the second electrode through which the electrical current passed from the bird. The water bath was raised so that the head was immersed and the bird was immediately stunned for 1 or 1.5 s using a set current of 70 or 100 mA (AC block) with 70 and 100 Hz, respectively. (Figure 1 and 2)

Prior to stunning each individual broiler was equipped with EEG and ECG electrodes. In order to facilitate the implantation of the electrodes, the broiler was hung in the restrainer.
The EEG electrodes (10 mm long and 1.5 mm in diameter; 55% silver, 21% copper, 24% zinc) were placed by pressing through the skin and skull: one electrode 0.3 cm to the right and one electrode 0.3 cm to the left of the sagittal suture and 0.5 cm of the imaginary transverse line at the caudal margin of the eyes. The ECG electrodes (35 mm long and 1.5 mm in diameter; same metal composition) were placed subcutaneously at the left and right side of the breast directly under the wing. The earth electrode for both the EEG and ECG was placed subcutaneously lateral at the right leg. The EEG and ECG were recorded from 30 s before and 2 minutes after stunning. The response of each animal to a pain stimulus (comb pinching) was observed for 2 minutes following the stun in order to assess unconsciousness. The birds were stunned again and immediately (<20 seconds) bled by neck cutting.

The recorder used was a DI 720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA).

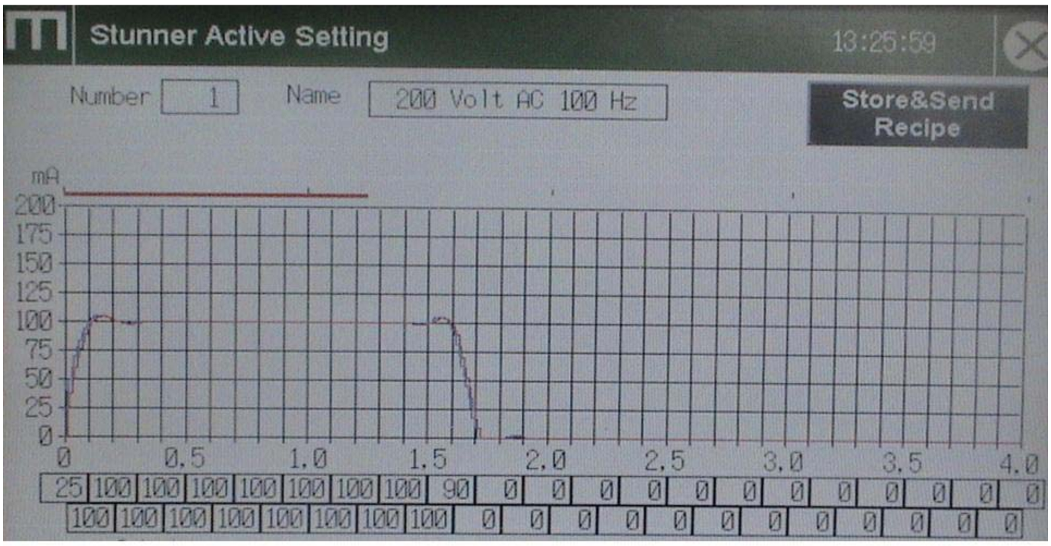

Figure 2: A controlled constant current.

2.2.3 Meat quality

A group of 60 broilers with an average live weight of 1.8 kg were selected from a group that was delivered to the slaughterhouse. Half of the group was stunned in the head cloaca stunner with the set electrical parameters of 100 mA, 100 Hz for 1.5 s and the others were used as controls and were stunned in the conventional water bath of the slaughterhouse with electrical parameters set at 100 mA, 100 Hz for 10 s). After stunning the broilers were slaughtered according to the regular slaughter procedure of the slaughterhouse.
Temperature, pH and colour in breast muscle (*P. major*) were measured after evisceration and chilling and pH at 1 day post mortem. Colour, cooking loss and shear force were also measured 4 days post mortem. Muscle pH and temperature was measured with knick pH meter connected to an Ingold electrode2 (Xerolyt, type LOT 406-M6-DXK-S7/25), and temperature with a Pt 1000 electrode.
Primary colour coordinates L*, a*, and b* were assessed [on CIE (Commission Internationale de l'Eclairage) LAB space] in the breast muscle (ventral side of *P. major*; presented as a mean value of the measurements in two locations equally distributed over
the muscle) using a Spectrofhoto meter Minolta CM-525i (Minolta, Osaka, Japan; light source D65). Chromatic values are given in the CIE-L*a*b* system. The L* value is a measure for lightness.
The a* and b* are the chromacity coordinates. The a* and b* indicate colour directions: +a* is the red direction, -a* is the green direction, +b* is the yellow direction and –b* is the blue direction.
Haemorrhages in breast (dorsal side of *P. major* and *minor*) and left and right thigh muscles (medial side) were quantified by a visual grading system (Veerkamp et al, 1987). The classification was performed independently by four observers. For classification, a threshold model consisting of a discontinuous five-point scale with four cut off points was used. Cut off points were formed by photographs of breast and thigh muscles showing a particular severity of haemorrhaging: Class 1 represents haemorrhage-free muscles and Class 5 represents muscles with numerous and severe haemorrhages (Lambooij et al, 1999).

2.2.4 Ethics

The experiments were approved beforehand by the Ethical Committee of the Animal Sciences Group of Wageningen UR.

2.2.5 Statistical analyses.

The EEG traces were subjected to correlation dimension (CD) analysis. This analysis provides a non-linear (fractal) measure of signal complexity (for algorithm see Broek et al., 2005). Correlation dimension analysis is a relatively new technique that has been customised to measure depth of anaesthesia in humans (Broek, 2003). The small amplitude, high frequency (awake) EEG signal is more complex than the large amplitude, low frequency (unconscious) EEG signal. Therefore, high CD values are taken to indicate awareness while low values indicate a state of unconsciousness. It is suggested that chickens are awake, drowsy and sleep at a CD score of 7, 6.6 and 6, respectively (Coenen and van den Broek, 2005). In poultry (McKeegan et al., 2007) it is suggested that a reduction in CD to 60% of the baseline value was an indicator of an unconsciousness during gas stunning.
Each bird represents an experiment with a probability P that the bird is unconscious during a general epileptiform insult. For n birds, treated independently, the number x, which are unconscious, is binomially distributied with total n and probability P. A confidence interval can be calculated for probability P based on a relationship between the binomial and beta distribution. The number of effective stuns follows a binomial distribution. A 95% confidence limit on the probability for an effective stun can be obtained by means of a well known relationship with the beta distribution (Johnson & Kotz, 1969).
An analysis of variance was used to analyse the heart rate and meat quality parameters (Genstat, 2008).

2.3 Results.

2.3.1 Brain and heart activities

Measurements with a controlled current of 100 mA were performed on 27 broilers weighing on average 2.2 ± 0.3 kg.
A general epileptiform insult with a tonic/clonic and exhaustion phase, followed by a mix of spikes, alpha, beta, theta and delta waves was observed on the EEG recordings. Seven birds were stunned for 1 s, three of which responded on the noxious stimulus 30 s after stunning and recovered rapidly according to the CD analyses (Broek 2003) (Figure 3). The other birds recovered slowly. (Figure 4). Because of the rapid recovery the following birds were stunned for 1.5 s. These birds may have been unconscious for approximately 20 s or more assuming that a CD score below 6 indicates unconsciousness. (Figure 5). Only 1 bird showed a positive noxious response after 60 s, 5 more birds displayed a response after 120 s. The behaviour showed one phase of tonic cramp followed by a phase of relaxation and 6 birds displayed wing flapping for several seconds (<10 s). Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=27), the chance of an effective stun for all broilers lies between 0.9 and 1.0 with a current of 100 mA (AC block) is used.
Minimal blood splashes were observed in carcasses of broilers stunned for 1s of which none in fillets and 3 in legs from 7 birds. In carcasses from 20 birds stunned for 1.5 s, blood splashes were seen in 4 fillets and 6 legs.

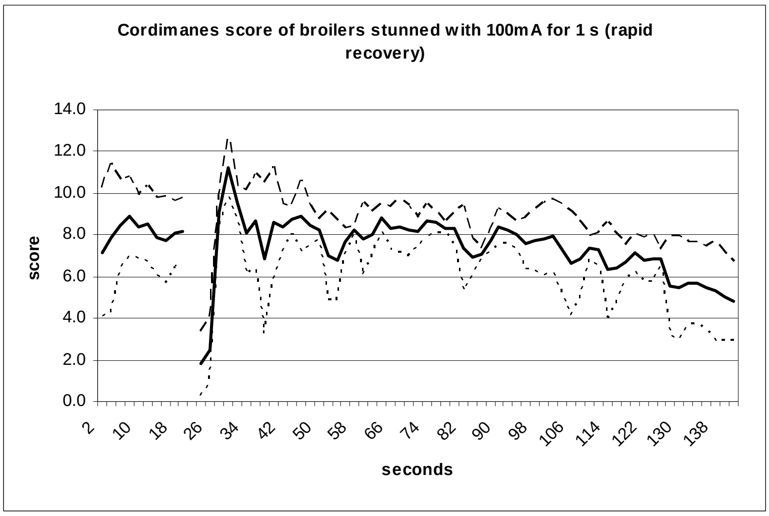

Figure 3: Cordimanes score from 3 broilers before and after head to cloaca stunning with a controlled current of 100 mA with a positive noxious response 30 s after stunning.

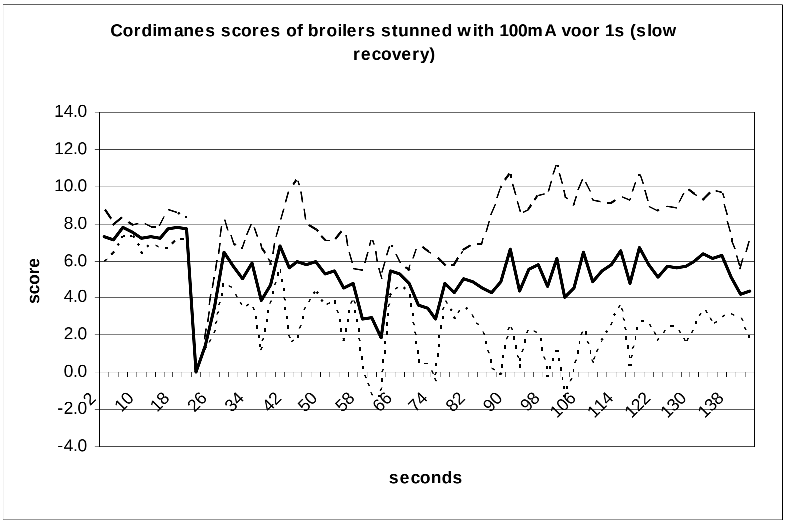

Figure 4: Cordimanes scores from 4 broilers before and after head to cloaca stunning with a controlled current of 100 mA.

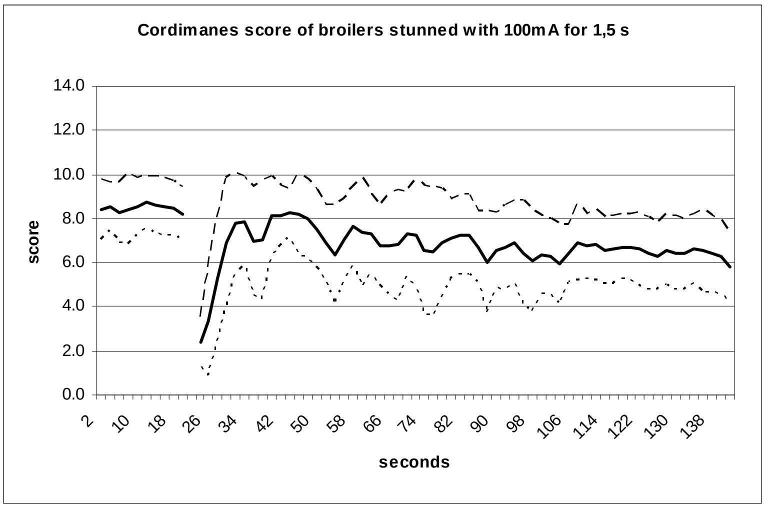

Figure 5: Cordimanes scores from 20 broilers before and after head to cloaca stunning with a controlled current of 100 mA.

A controlled current of 70 mA was performed with 28 broilers weighing on average 2.3 ± 0.3 kg. Eleven and 17 broilers were stunned for 1 and 1.5 s, respectively. A general epileptiform insult with a tonic/clonic and exhaustion phase, followed by a mix of spikes, alpha, beta, theta and delta waves was observed on the EEG recordings.
After stunning 2 broilers stunned for 1 and 1.5 and 9 stunned for 1.5 s responded to a noxious stimulus 30, 60 and 120 s, respectively, after stunning. According to the CD analyses (Figure 6 and 7) . These birds may have been unconscious for approximately 15 to 20 s or longer.
Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=28), the chance of an effective stun for all broilers lies between 0.9 and 1.0 when a current of 70 mA (AC block) is used.
Minimal blood splashes were observed in carcasses of broilers stunned for 1s, of which none in fillets and 1 in leg muscle from 11 birds and for those stunned for 1.5 s there were 3 in fillets and 9 in legs from 17 birds.

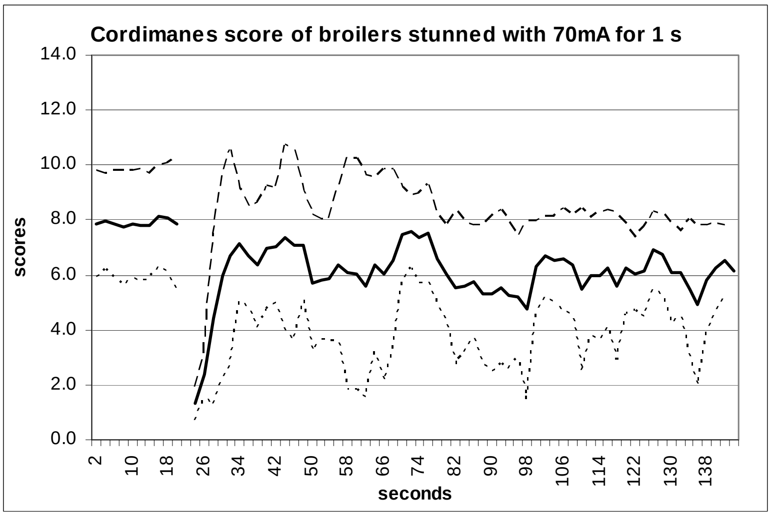

Figure 6: Cordimanes scores from 11 broilers before and after head to cloaca stunning with a controlled current of 70 mA.

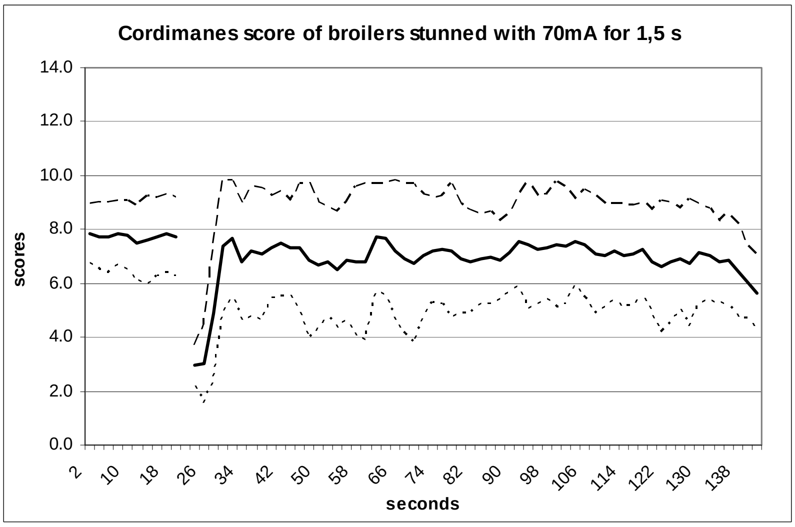

Figure 7: Cordimanes scores from 17 broilers before and after head to cloaca stunning with a controlled current of 70 mA.

The average heart rate prior to stunning with 70 and 100 mA was 387 ± 45 and 358 ± 51 beats/min, respectively. After stunning the ECG revealed fibrillation for 15± 4 and 14 ± 6 s, respectively. The heart rate decreased significantly ($p < 0.05$) after stunning and recovered afterwards (Figure 8).

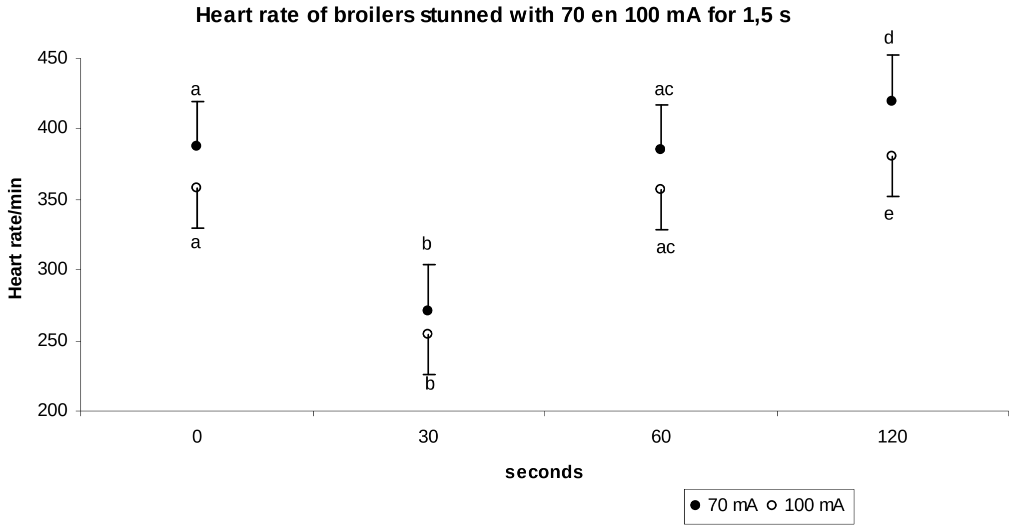

Figure 8: Heart rate in beats/min prior and after stunning with 70 and 100 mA. A different super- or underscript means a significant difference ($P < 0.05$).

2.3.2 Meat quality

The results of the meat quality parameters are presented in Tables 1 and 2. The pH after chilling is significantly ($p < 0.05$) higher in the head cloaca group and the shear force lower ($p < 0.01$), which suggests that the meat is tender. The percentage of fillets and legs with no blood splashes is higher in the head-cloaca group, with fewer severe blood splashes or none in the carcasses from head cloaca stunned birds compared to those stunned in the commercial water bath.

Table 1: Results of the meat quality parameters measured in the fillets after chilling, 1 and 4 days post mortem in carcasses of broilers stunned in a commercial water bath (100 mA, 100 Hz, 10 s) head cloaca (100 mA, 100 Hz for 1.5 s) (Statistical significance * $p < 0.05$; ** $p < 0.01$).

	Water bath	Head cloaca
Carcasses N	28	26
T after chilling °C	7.54 ± 0.79	7.47 ± 0.84
pH after chilling	6.18* ± 0.21	6.56* ± 0.22
pH 1 D p.m.	6.11 ± 0.12	6.13 ± 0.16
Cooking loss %	11.5 ± 2.11	11.7 ± 2.39
Shear force (N.)	27.5** ± 11.9	20.9** ± 11.7
L* after chilling	58.0 ± 2.24	58.0 ± 1.76
a* after chilling	4.7 ± 0.91	4.7 ± 0.90
b* after chilling	11.7 ± 1.66	11.8 ± 1.29
L* 4 D p.m.	58.3 ± 2.25	59.6 ± 1.91
a* 4 D p.m	4.9 ± 0.96	4.5 ± 0.80
b* 4 D p.m	13.7 ± 1.56	14.3 ± 1.16

Table 2: Percentage of blood splashes in the fillets and legs according to score of 1 to 5 (score 1 = none and 5 = severe) in carcasses of broilers stunned in the water bath or head-cloaca

		1	2	3	4	5
Water	Fillets %	7	29	36	21	7
bath	Legs %	34	27	20	13	7
Head	Fillets %	23	35	27	15	0
cloaca	Legs %	27	23	17	25	8

2.4 Discussion.

If sufficient current is administered through the brain of an animal a general epileptiform insult or grand mal seizure (all brain parts are stimulated) will occur (EFSA, 2004). The epileptic process is characterised by rapid and extreme depolarisation of the membrane potential and there is heterogeneity of findings (Kooi et al., 1978). As measured on the EEG such an insult consists of relatively small waves increasing in amplitude in the tonic phase, and decreasing in frequency in the clonic phase resulting ultimately in a period of strong depression of electrical activity (Lambooy, 1982). A human being is unconscious during the three phases of a general epileptiform insult. Moreover, the brain is in a stimulated condition and unable to respond to additional stimuli. By analogy, a mammal is assumed to be also unconscious and insensible (Lopes da Silva, 1983). It is suggested that the depth and duration of unconsciousness induced by electrical stunning is determined by the duration of the RMS current as opposed to peak current level (Raj & O’Callaghan, 2004). In our study the current and frequency used seem to have been sufficient to induce epileptiform activity instantaneously.
The behaviour showed one phase of tonic cramp followed by relaxation and in a few cases also wing flapping. The heart failure results in a gradual reduction in blood pressure and lack of oxygen to the brain and affects the characteristics of general epileptiform insult (Savenije et al., 2002). Disorder is observed in our study using a lower current of 70 and 100 mA and a higher frequency of 70 and 100 Hz using a AC block waveform. At frequencies between 200 and 1600 Hz a higher current is necessary to induce the seizure. A consequence is that the duration of the insult is shorter (Raj & O’Callaghan, 2004; Raj et al., 2006). It is suggested that the cloaca electrode resulted in alternative and better pathways to stimulate the brain and heart. However, in this equipment the broilers recovered which was not the case in a previous experiment (Lambooij et al, 2008b). According to the correlation dimension scores the broilers recovered after stunning but displayed unconsciousness and showed delta, spikes with no response to a noxious stimulus which may indicate that the brain is in a stimulated condition (Figures 5, 6 and 7) . When the birds were bled immediately after the stun, they remained unconscious. When they were stunned with 100 mA for 1.5 s the birds remained stunned for a longer period which may have prevented them for returning to a conscious state during exsanguination, which is supported by the study of Hindle et al. (2010).
In a conventional water bath, in which the electrical current is applied through the whole body, a minimum current of 120 mA per bird is recommended to induce unconsciousness and a cardiac arrest (Gregory & Wotton, 1990). This recommended minimum current for broilers in the EU increases quality defects (haemorrhages, broken bones) of carcasses and broiler meat (Gregory & Wilkins, 1989).
Shackling involves hanging live birds upside down, suspended by their feet and the restrained legs must bare the body weight of the broilers. Electrical water bath stunning of broilers has the most detrimental effect with respect to muscle haemorrhaging (Lambooij et al., 1999). Haemorrhages can be induced by stunning, however, the underlying mechanism is considered to be multi factorial (Kranen et al., 1996). The validity of the present recommendations set at 100 mA for broilers stunned at a frequency of 50 Hz with the result that more than 67 % of the carcasses displayed blood splashes has been questioned (Hindle et al, 2010). Moreover, it became clear that effective stuns involving increasing frequencies required higher currents to produce effective pre-slaughter stunning, while increasing the risk of blood splashing in muscle tissue. Stuns performed at higher current levels are almost certain to result in haemorrhaging. Therefore, the challenge still remains of providing an alternative stunning method with an effective threshold current that will induce consciousness and insensibility in broilers without compromising carcass quality (Hindle et al., 2010) . When broilers are stunned head to cloaca they can be effectively stunned with minimal effect on quality defects, which

became apparent during a previous study (Lambooij et al., 2008) and was supported by results from the present experiments.

2.5 Conclusions

It can be concluded from this experiment that broilers are effectively stunned with a controlled current of 70 (70 Hz) and 100 mA (100 Hz; AC block)) for 1 or 1.5 s using a water bath where the head of the broiler is immersed in the water as electrode and a steel electrode at the cloaca as opposite electrode. Since the broilers may recover rapidly during 70 mA for 1 s 100 mA for 1.5 s is recommended to safeguard that the bird remains unconscious during exsanguination. The meat in the fillet is potentially tender and there are fewer incidences of blood splashes in both fillets and legs of carcasses head cloaca stunned compared to those stunned in a conventional water bath.

3 Head only electrical stunning.

3.1 Introduction.

Stunning of slaughter animals is in the first place applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover while bleeding to death (exsanguination). Secondly, stunning should produce sufficient immobility to facilitate the initiation of exsanguination (Blackmore and Delany, 1988). It is generally stated that unconsciousness and insensibility should be induced as soon as possible and without a detrimental effect on the welfare of the animal and the meat quality of the carcass.
According to the EU Council Directive of 1993 on the protection of animals at the time of slaughter it is stated that horses, ruminants, pigs, rabbits and poultry brought into abattoirs for slaughter shall be a) moved and if necessary lairaged, b) restrained and c) stunned before slaughter. Animals must be restrained in an appropriate manner, so as to spare them any avoidable pain, suffering, agitation, injury or contusions. Animals must not be suspended before stunning or killing. However, poultry and rabbits may be suspended for slaughter provided that appropriate measures are taken to ensure that they are in a sufficiently relaxed state for stunning. Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to special gas mixtures.
Electrical and gas stunning are generally used to induce unconsciousness during cutting and bleeding for reasons of animal welfare in the EU or to induce immobilisation to facilitate automatic neck cutting in the USA. The Treaty of Amsterdam (EFSA – AHAW/04-027, 2004) set new rules for the EU in a “Protocol on the Protection and Welfare of Animals”. It recognizes the sentience of animals and requires that European Institutions pay full regard to the welfare requirements of animals in the formulation and implementation of Community regulations. Animals should be protected from any anthropogenic excitement, pain or suffering during transport, lairaging, restraint, stunning, slaughter or killing. Broilers stunned at 50 Hz can be effectively stunned at between 45 – 240 mA of which 39% received a current below the recommended 100 mA (EFSA, 2004) making it difficult to determine the threshold current necessary to induce an epileptiform EEG (Raj et al., 2006; Hindle et al., 2010).
The most common electrical stunning method for animals uses a frequency of 50 Hz alternating current (AC.) with sinusoidal waveform. The frequency can be as high as 1800 Hz (Anil & McKinstry, 1992; Lambooij et al., 1997) and the waveform can be square or rectangular. High frequency electrical stunning can induce epilepsy in the brain. However, the durations are shorter than those with 50 Hz. During the conventional water bath stunning of broilers, electrical current passes through the whole body, which results in direct muscle stimulation. Super contraction, movement between muscles and abnormal position of the animal during stunning may cause blood vessels to rupture and muscle fibres to be damaged (Hillebrand et al., 1999; Kranen et al., 2000). An alternative to whole body electrical stunning is head-only stunning, where the stunning current passes only through the head of the animal. Head only stunning of broilers using 50 Hz, 117 V, corresponding with 336 mA per bird, appeared to be effective (Gregory & Wotton, 1990).
The objective of the study was to evaluate the behavioural, neural and physiological responses of broilers after head-only electrical stunning restrained by their feed and body in a cone. Meat quality after head only electrical stunning was compared with the currently applied conventional water bath method in a commercial setting. Analysis of pH, colour, cooking losses and shear force was performed in fillets during storage and scoring of blood splashes were chosen as parameters for product quality evaluation.

3.2 Materials and methods

3.2.1 Animals.

Forty-seven broilers delivered at the slaughterhouse from a commercial farm were used for these experiments. Before transport the animals had a feed withdrawal period of 6 h and were transported in crates. During the experiment the birds were placed one by one in a specially developed cone shaped restrainer and stunned using a head-only controlled current stunner. (Figure 9) The birds were hung

individually by the feet from shackles and electrically stunned via three pointed electrodes on both sides of the head. The stunning current ran through the head for 0.5 to 5 s.
After the stunning experiment the broilers were weighed and breast and leg muscles were visually controlled for blood splashes (yes or no).

3.2.2 Experimental design

Each broiler was hung individually by the feet from the shackles and the body rested in a raising cone where the head was fixed by the electrodes on both sides of the head outside the cone. (Figures 9 and 10. Tiel Engineering BV, Tiel Netherlands). Immediately the current was passed through the head for 1 to 5 s. The electrical parameters were set at 240 mA, 50 Hz and a sinusoidal AC wave form.

Prior to stunning each individual broiler was equipped with EEG and ECG electrodes. In order to facilitate the implantation of the electrodes, the broiler was hung by the feet in the restrainer.
The EEG electrodes (10 mm long and 1.5 mm in diameter; 55% silver, 21% copper, 24% zinc) were placed by pressing through the skin and skull: one electrode 0.3 cm to the right and one electrode 0.3 cm to the left of the sagittal suture and 0.5 cm of the imaginary transverse line at the caudal margin of the eyes. The ECG electrodes (35 mm long and 1.5 mm in diameter; same metal composition as above) were placed subcutaneously at the left and right side of the breast directly under the wing. The earth electrode for both the EEG and ECG was placed subcutaneously lateral at the right leg. The EEG and ECG were recorded from 30 s before to 2 minutes after stunning. The response of each animal to a pain stimulus (comb pinching) was observed for 2 minutes following the stun in order to assess unconsciousness. The birds were stunned again and immediately (<20 seconds) bled by neck cutting.

The data recorder used was a DI 720 data recording module with a WinDaq Waveform browser (Dataq Instruments, Akron, Ohio, USA

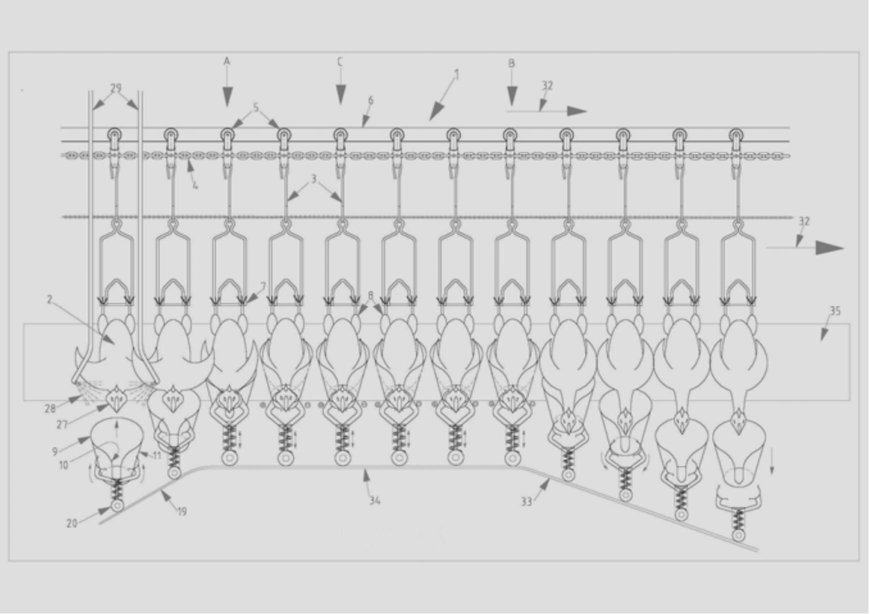

Figure 9: Restraining of the broilers by there feet and by there body in a raising cone.

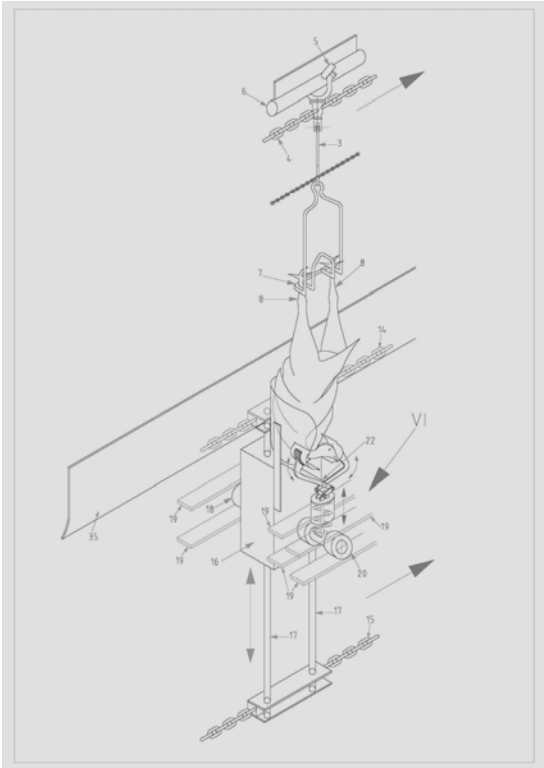

Figure 10: The head is fixed by the electrodes outside the cone. Upon contact the current passes immediately through the head.

3.2.3 Meat quality

A group of 50 broilers with an average live weight of 2.2 kg were selected from a group that was delivered to the slaughterhouse. Half of the group was stunned in the head-only stunner with the electrical parameters set at 240 mA, 50 Hz for 5 s and the remaining birds were used as controls stunned in the conventional water bath of the slaughterhouse with the electrical parameters set at 100 mA, 100 Hz for 10 s). After stunning the broilers were slaughtered according to the regular slaughter procedure of the slaughterhouse.
Temperature, pH and colour in breast muscle (*P. major*) were measured after evisceration and chilling and pH at 1 day post mortem. Colour, cooking loss and shear force were also measured 4 days post mortem. Muscle pH and temperature was measured with knick pH meter connected to an Ingold electrode2 (Xerolyt, type LOT 406-M6-DXK-S7/25), and temperature with a Pt 1000 electrode. Primary colour coordinates L*, a*, and b* were assessed [on CIE (Commission Internationale de l'Eclairage) LAB space] in the breast muscle (ventral side of *P. major*; presented as a mean value of the measurements in two locations equally distributed over
the muscle) using a Spectrofhoto meter Minolta CM-525i (Minolta, Osaka, Japan; light source D65). Chromatic values are given in the CIE-L*a*b* system. The L* value is a measure for lightness. The a* and b* are the chromacity coordinates. The a* and b* indicate colour directions: +a* is the red direction, -a* is the green direction, +b* is the yellow direction and –b* is the blue direction.
Haemorrhages in breast (dorsal side of *P. major* and *minor*) and left and right thigh muscles (medial side) were quantified by a visual grading system (Veerkamp et al, 1987). The classification was performed independently by four observers. For classification, a threshold model consisting of a discontinuous five-point scale with four cut-off points was used. Cut-off points were formed by photographs of breast and thigh muscles showing a particular severity of haemorrhaging: Class 1 represents haemorrhage-free muscles and Class 5 represents muscles with numerous and severe haemorrhages (Lambooij et al, 1999).

3.2.4 Ethics

The experiments were approved beforehand by the Ethical Committee of the Animal Sciences Group of Wageningen UR.

3.2.5 Statistical analyses.

The EEG traces were subjected to correlation dimension (CD) analysis. This analysis provides a non-linear (fractal) measure of signal complexity (for algorithm see Broek van den et al., 2005). Correlation dimension analysis is a relatively new technique that has been customised to measure depth of anaesthesia in humans (Broek van den, 2003). The small amplitude, high frequency (awake) EEG signal is more complex than the large amplitude, low frequency (unconscious) EEG signal. Therefore, high CD values are taken to indicate awareness while low values indicate a state of unconsciousness. It is suggested that chickens are awake, drowsy and asleep at a CD score of 7, 6.6 and 6, respectively (Coenen and van den Broek, 2005). In poultry (McKeegan et al., 2007) suggested that a reduction in CD to 60% of the baseline value can be as an indicator of an unconsciousness during gas stunning. Each bird represents an experiment with a probability P that the bird is unconscious during a general epileptiform insult. For n birds, which are treated independently, the number x, which are unconscious, is binomially distributed with total n and probability P. A confidence interval can be calculated for probability P based on a relationship between the binomial and beta distribution. Therefore, the number of effective stuns follows a binomial distribution. A 95% confidence limit on the probability for an effective stun can be obtained by means of a well known relationship with the beta distribution (Johnson & Kotz, 1969).
An analysis of variance was used to analyse the heart rate and meat quality parameters (Genstat, 2008).

3.3 Results

3.3.1 Brain and heart activities

The results of the different parameters measured are presented in Table 3. A general epileptiform insult with a tonic phase was followed by a clonic and exhaustion phase followed by recovery as observed on the EEG recordings. (Figure 11). The CD score analyses are presented in Figures 12, 13 and 14. During stunning the broilers displayed wing flapping, which was intensive in 5 birds. Within a confidence limit of 95%, taking into account the number of animals with a reliable EEG (n=47), the chance of an effective stun with all broilers lies between 0.95 and 1.00 when an average current of 190 ± 30 mA (sinusoidal AC) for 0.5 s is used.
Minimal blood splashes were observed carcasses of broilers stunned for 0.5s in 3 fillets and 2 legs of 21 birds and no blood splashes were observed in fillets or legs from 26 birds stunned for 3 and 5 s. Broken wings were observed after 0.5, 3 and 5 in 3, 1 and 2 carcasses, respectively

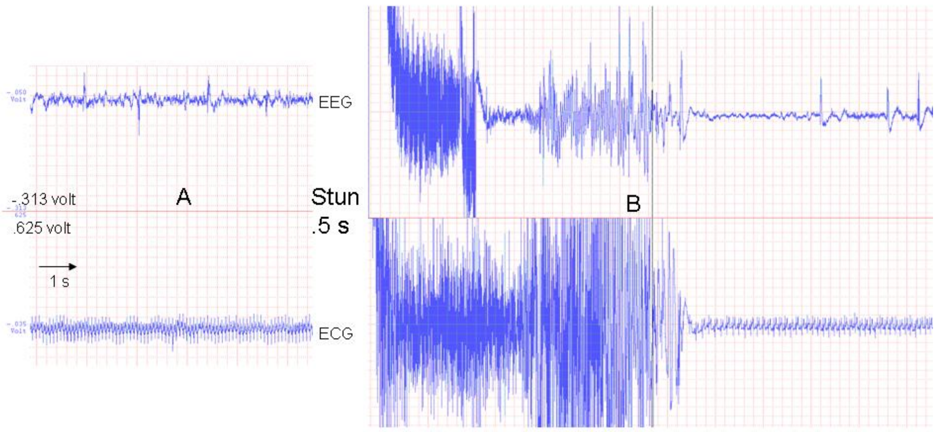

Figure 11: EEG and ECG recording before and after stunning. Prior to stunning a normal EEG and ECG rhythm can be observed (A). After stunning a tonic phase is followed by a clonic phase and exhaustion followed by recovery on the EEG and heart fibrillation followed by recovery on the ECG (B).

Table 3: Results of the neural and physiological parameters when broilers were stunned electrically stunned head only. A different superscript in the column is significance $p < 0.05$ according the ANOVA.

Stunning	Duration s	0.5	3	5
	Number N	21	14	12
	Weight kg	2.2 ± 0.2	1.5 ± 0.2	1.8 ± 0.2
	Current mA	190 ± 30	320 ± 37	313 ± 24
EEG	insult s CD score s	30 ± 5 18	44 ± 11 12	65 ± 19 16
Positive noxious stimulus	After stunning 30 s N 60 s N 120 s N	 2 7 13	 0 2 11	 0 1 6
ECG	Fibrillation s	18 ± 4	13 ± 4	12 ± 3
Heart rate	Prior stunning After stunning 30 s rate beats/min 60 s rate beats/min 120 s rate beats/min	416 ± 36[a] 311 ±5[b] 404 ± 52[a] 429 ± 33[a]	416 ± 36[a] 277 ± 53[b] 348 ± 58[c] 408 ± 47[a]	404 ± 39[a] 243 ± 48[b] 355 ± 44[c] 434 ± 42[a]

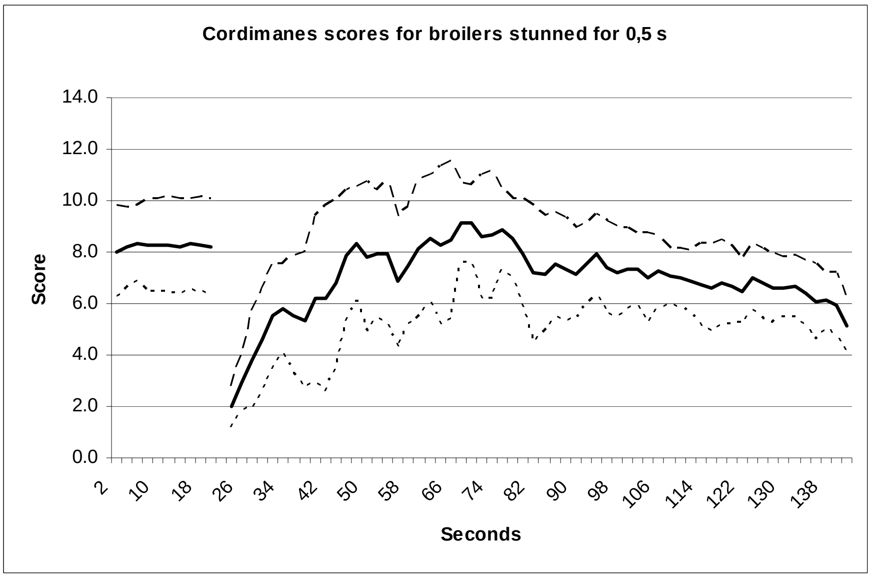

Figure 12: Cordimanes score of 21 broilers before and after head only stunning with a current of 190 ± 30 mA for 0.5 s.

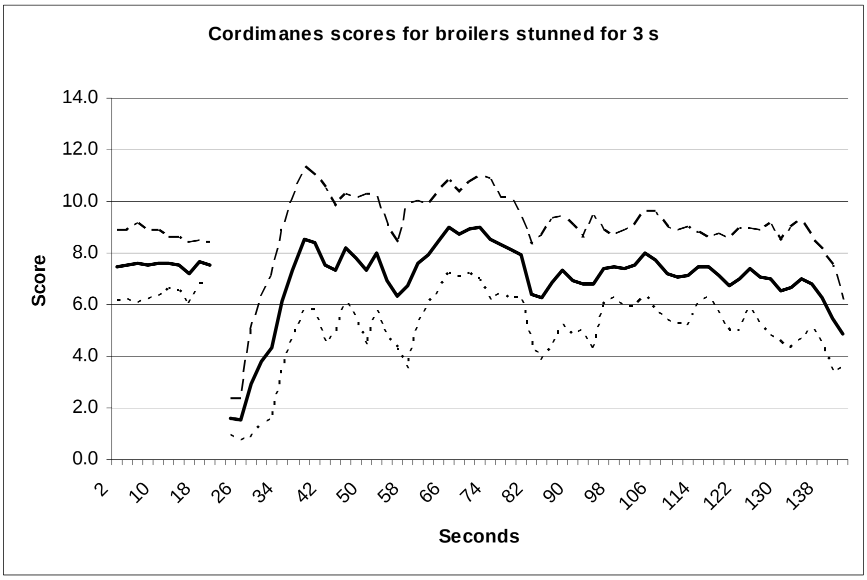

Figure 13: Cordimanes score of 14 broilers before and after head only stunning with a current of 320 ± 37 mA for 3 s.

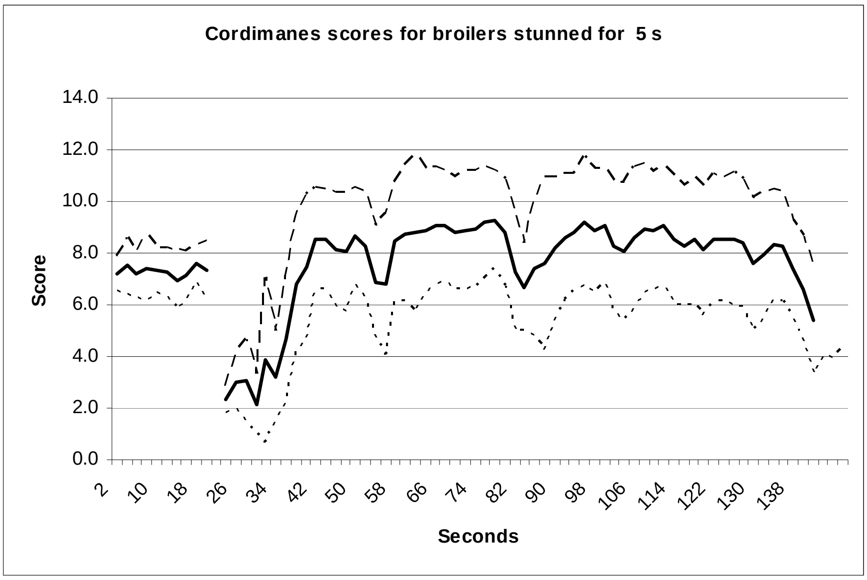

Figure 14: Cordimanes score of 14 broilers before and after head only stunning with a current of 313 ± 24 mA for 3 s.

3.3.2 Meat quality

The results of the meat quality parameters are presented in Tables 4 and 5. The pH after chilling is significantly ($p < 0.05$) lower in the head only group (table 4). The percentage of fillets with no blood splashes is 80% (head-only) compared to 16% after conventional stunning and with no severe blood splashes in the carcasses stunned head only compared to 12% for those stunned in the commercial water bath. Blood splashing in the legs was more severe in the conventional water bath.

Table 4: Results of the meat quality parameters measured in the fillets after chilling, 1 and 4 days post mortem in carcasses of broilers stunned in a commercial water bath (100 mA, 100 Hz, 10 s) and head only (240 V, 50 Hz for 5 s) (Statistical difference * $p < 0.05$).

	Water bath	Head only
Carcasses N	25	25
T after chilling °C	8.05 ± 1.34	8.05 ± 1.34
pH after chilling	6.62* ± 0.26	6.00* ± 0.15
pH 1 D p.m.	5.94 ± 0.20	5.91 ± 0.14
Cooking loss %	12.7 ± 2.96	14.1 ± 1.79
Shear force (N.)	36.4 ± 21.17	36.6 ±27.2
L* after chilling	58.8 ± 1.97	60.2 ± 1.71
a* after chilling	4.1 ± 0.96	3.3 ± 0.74
b* after chilling	11.1 ± 1.56	10.0 ± 1.56
L* 4 D p.m.	60.3 ± 2.38	61.7 ± 1.38
a* 4 D p.m	4.7 ± 1.33	4.0 ± 0.70
b* 4 D p.m	14.5	14.2 ± 1.47

Table 5: Percentage of blood splashes in the fillets and legs according a score of 1 to 5 (score 1 = none and 5 = severe) in carcasses of broilers stunned in the water bath or head only

		1	2	3	4	5
Water	Fillets %	16	8	16	48	12
bath	Legs %	6	8	30	28	28
Head	Fillets %	80	4	8	8	0
only	Legs %	16	8	16	48	12

3.4 Discussion

The insight into the stunning process that has come from neural and physiological studies is very important. Assessment of more parameters than the general epileptiform insult and the analgesia may support the humaneness of the stunning and killing system. EEG, rhythm analyses (FFT, CD), brain tissue impedance (ECV) and neurotransmitter release measurements have been used to assess the effectiveness of electrical head-only stun duration on welfare. The minimum root mean square (RMS) sine wave currents necessary to head-only stun chickens were found to be 240 V using stunning electrodes with 3 pins. (Gregory and Wotton, 1990). When prolonged administration of currents (minimum of 5 sec) were used neck cutting by severing all the major blood vessels in the neck within 10 to 15 sec from the end of stun prevented the return of consciousness in these birds. Other EEG studies demonstrated that a lower current level of 134 mA (25 V, 150 Hz, 1 s) resulted in a general epileptiform that lasted 32 s. The reduction in effectiveness of current might be due to the impaired ability of the stun current to penetrate the skin (Hillebrand et al., 1993).
The method of recording the ECV as a measure for change in the extracellular volume is found to be a valid model in ischaemia-induced brain damage experiments in broiler chickens (Ruis-Heutinck et al, 1998). Animals that were bled only decreased from base extracellular volume after four minutes post mortem, while electrical head-body stunning, inducing cardiac fibrillation, causes an immediate and gradually increasing change in brain impedance. This suggests that the last method provides an adequate stun. Head-only stunning with exsanguination caused a duality in the response patterns found. Some animals showed a response similar to that of animals bled only and some animals similar to that of head-body stunning (Savenije et al., 2000, 2002).
CD analysis is a relatively new technique that has been customised to measure depth of anaesthesia in humans (Broek, van den 2003). The small amplitude, high frequency (awake) EEG signal is more complex than the large amplitude, low frequency (unconscious) EEG signal. Therefore, high CD values are taken to indicate awareness while low values indicate a state of unconsciousness. Earlier studies with chickens suggested that they were asleep at a score of 6 and during gas stunning a reduction in CD to 60% of the baseline value was seen as indicator of an unconsciousness level similar to anaesthetized humans (Coenen & van den Broek, 2005; McKeegan et al., 2007). When these scores are used for electrical head-only stunning, as in our experiment, the duration of insult is 12 to 18 s after stunning. On the basis of a visual score the duration of unconsciousness is scored from 30 to 65 s after stunning, moreover only 2 broilers responded at 30 s after a 0.5 electrical stun on a noxious stimulus and none after a 3 or 5 s stun.
Therefore, it can be recommended to start exsanguination immediately after stunning, Gregory & Wotton (1990a,b) suggested neck cutting by severing all the major blood vessels in the neck within 10 to 15 s from the end of the stun to prevent return to consciousness. Combining head-only stunning with exsanguination has a synergistic affect on the release of glutamate and aspartate, which increases the duration of unconsciousness. Sticking following a stun should be carried out as promptly as possible when using head-only stunning as the duration to loss of brain responsiveness varies for different species following sticking (Cook et al, 1996) .In the case of our equipment the bird can be neck cut immediately after stunning, because the head is restrained by the electrodes (Figure 10).
During a recent study (Hindle et al, 2010) broilers stunned at 50 Hz died in the range 45-220 mA, 39 % of which were stunned below the EFSA recommended level (EFSA, 2004). As frequency increased to 400 Hz 48% of broilers died after stuns above recommended levels, 86% of the stuns below the EFSA recommendation resulted in periods of unconsciousness (i.e. recovery within 1 minute) that were unacceptable for effective stunning. Stuns with broilers performed at a frequency of 1000 Hz gave 50% unacceptable results of which 36% were performed below EFSA recommendations, which is for head only stunning 240 V for 5 s to prolong the duration of unconsciousness (Gregory & Wotton, 1990a,b). All the broilers in our study showed a general epileptiform insult stunned with 0.5, 3 or 5 s, however the duration was not prolonged with an increasing stunning duration according the CD scores and prolonged according the visual scoring. In contrast, stunning in a single-bird water bath the broilers as in our equipment may improve the chance of an effective stun for each bird.

Haemorrhaging results in a decrease in quantity (trimming) and quality of poultry products, and hence causes economical losses to the poultry industry. Therefore haemorrhaging, in particular of the valuable breast meat, is considered a major quality defect. Haemorrhages can be induced by stunning, however, the underlying mechanism is considered to be multi-factorial (Kranen et al., 1996).

The morphology of haemorrhages investigated was dependent on the tissue in which they occurred. In the pectoral muscles extravasating blood was found to follow the direction of the muscle fibres. In fat tissue, the majority of the haemorrhages had a petechial appearance. More diffuse haemorrhages were found in loose connective tissue. (Kranen et al., 2000).

More than 67 % of the carcasses from broilers stunned at 50Hz displayed blood spots, 85% of which were from broilers that died during the stunning procedure. Stunning at 400 Hz produced 35% of carcasses with blood spots of which 81% were from broilers that died during the stunning procedure. Stunning at 1000 Hz resulted in 18% of carcasses with blood spots of which 88 % were from broilers that died during the stunning procedure. Although there appears to be a tendency towards fewer incidences of blood splashing as frequency increases, the stun to kill policy does not appear to improve the situation (Hindle et al, 2010). A higher incidence of wing damage after gas stunning, compared to electrical stunning, has been observed in a study of Raj et al. (1992). Wing damage in that study was due to the severe convulsions caused by gaseous stunning. Gas killing of broilers substantially reduces the incidence of broken bones and haemorrhaging in breast muscles in association with or without the broken bones (Raj, 1997; Uijttenboogaart, 1997) During head only stunning in our experiments the broilers show wing flapping during stunning, which was sometimes intensive. This wing flapping may be the cause of the broken wings which may induce haemorrhages. This is a disadvantage of head only stunning. As seen in our experiments, however there was a large difference in the fillets of carcasses of broilers stunned head only. In 80 % of the fillets of broilers stunned head only showed no blood splashes and severe blood splashes were not observed, whereas only 16% of those stunned in the conventional water bath group were free of blood splashes and 12% displayed severe haemorrhages.

3.5 Conclusions

It can be concluded that, broilers may be insensible and unconscious after head-only electrical stunning with pinned electrodes using an average current of 190 ± 30 mA (sinusoidal AC) for 0.5 s. For practical implementation a set current of 250 mA (average + 2*SD) is recommended to overcome individual differences in resistance. To prevent recovery the stun should be followed by an immediate neck cut. Since carcass quality is limited compromised such equipment should be further developed for practical application and commercial use.

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