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Report 398

Report on restraining and neck cutting or stunning and neck cutting in pink veal calves

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Abstract
Neural and physiological assessment of welfare during restraining and rotation, after neck cutting, neck cutting followed by captive bolt stunning and electrical stunning followed by neck cutting in pink veal calves

Keywords
Cattle, neck cutting, stunning, mechanical, electrical, welfare

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Dit onderzoek is uitgevoerd in opdracht van het Ministerie van Landbouw, Natuurbeheer en voedselkwaliteit. BO-07-011-038-004 Bedwelmd ritueel slachten van runderen volgens de Islamitische ritus

Preface

According to the Dutch legislation (Gezondheids- en Welzijnswet, 1993; Besluit ritueel slachten), the EU Council Directive (1993) and future Council Regulation (2009) on the protection of animals at the time of killing 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. The Directive makes an exception for omitting stunning during ritual slaughter. The different religious groups have established their own rules. There are many different rules used in Halal slaughter. This variation is due to different interpretations of the Koran and Hadjis. The first step for slaughter is restraining the animal in the conventional restrainers in the slaughterhouse. After restraining the animal is exsanguinated by cutting the major blood vessels in the neck.

The question remains whether or not the animal suffers during exsanguination. It is difficult to measure pain in an animal and results from different experiments show a different duration of consciousness after the neck cut. The results of a minimal anesthesia model in calves showed that the impact of ventral-neck incision was associated with significant noxious sensory input that would have been likely to be perceived as pain in conscious animals. In cattle the duration is longer compared to other species due to the anastomes of the aa. vertebrales and occurrence of ballooning or false aneurisms during bleeding.

The government should establish requirements for handling during ritual slaughter and facilitate a dialogue with those religious groups involved.

Samenvatting

Inleiding

Internationale en nationale wetgeving bepalen dat slachtdieren voorafgaand aan het doden door verbloeden op een adequate manier moeten worden verdoofd, waarbij een uitzondering wordt gemaakt voor ritueel slachten. Voor het verdoven dienen de dieren zodanig te worden gefixeerd, dat pijn, lijden, agitatie of beschadiging wordt vermeden. Met een adequate verdoving wordt een methode bedoeld, die zonder aanvaardbare opwinding of pijn lijdt tot bewusteloosheid, gevolgd door een dodingshandeling die leidt tot de dood. Toegestane methoden voor het verdoven zijn schietmasker, kopslag, elektronarcose en gassen. Wanneer dieren zonder bedwelming worden gedood moet gecontroleerd worden dat het dier bewusteloos is voordat het uit de fixatie mag worden vrij gelaten. Het doel van het onderzoek is het effect op de hersen- en hart activiteit te beoordelen gedurende fixatie en 90^{o}, 120^{o} of 180^{o} draaien en vervolgens wel of niet bedwelmen voor het verbloeden. Tevens zijn bloed waarden gerelateerd aan de energievoorziening gemeten.

Materiaal en methoden

Eenendertig rosé vlees kalveren afkomstig van verschillende mest bedrijven werden voor het onderzoek gebruikt. Het waren rood- en zwartbonte dieren van een gemengd ras met een warm slachtgewicht van gemiddeld 185 ± 26 kg. De dieren werden in de wachtruimte geselecteerd, uit de staart bloed getapt en uitgerust met EEG en ECG apparatuur. Daarna werden zij naar de verdovingsplaats gedreven, bloed getapt en in het kantel apparaat gedreven. Na fixatie van de kop werden de kalveren 90^{o}, 120^{o} of 180^{o} gedraaid en werd direct de hals ingesneden, de hals ingesneden gevolgd door verdoven met het schietmasker of elektrisch verdoofd en de hals ingesneden voor het verbloeden.

Resultaten

Na het insnijden van de hals vertoonde de CD (Correlatie Dimensie) score een langzame daling evenals het %power van de hogere frequenties (bewustzijn). De oogreflex verdween 135 ± 57 s na toediening van de halssnede. De regressie coëfficiënt R tussen de CD score en het %power was 0,6 ($P < 0.01$). Na het verdoven met het schietmasker na de halssnede daalt de CD score en het power % sterk. De R tussen beide parameters was 0.8 ($P< 0.01$) Dit was ook het geval als de kalveren elektrisch werden verdoofd gevolgd door de halssnede. De R was 0.4 ($P < 0.01$)
De hartslag in de wachtruimte na het aanbrengen van de ECG elektroden was 84 – 92 slagen/min en rees significant ($p<0.05$) naar 113 -118 slagen/min na drijven naar de fixatie apparatuur en rees verder naar 126 – 150 slagen/min tijdens 90^{o}, 120^{o} of 180^{o} draaien. De hartvariabiliteit veranderde sterk tijdens draaien in die zin dat de L/R waarde (maat voor stress) daalde naar een erg lage waarde ($p<0.05$).
Belangrijke verhogingen in de bloedwaarden werden aangetoond in de zuurstof druk tussen wachtruimte en ingang van het kantelapparaat en in de BE waarde (maat voor verzuring) na 180^{o} draaien.

Conclusies

Door het draaien tijdens de fixatie steeg de hartslag en veranderde de hartvariabiliteit (daling L/R waarde), waardoor het welzijn mogelijk wordt geschaad. Er was geen verschil in rotatie van het dier tot 90^{o}, 120^{o} of 180^{o}.

Bewusteloosheid trad bij de rosé vleeskalveren op
na gemiddeld ongeveer 80 s na het toedienen van alleen de halssnede,
4 s na de halssnede door verdoving met het schietmasker
voor het toedienen van de halssnede na elektrische bedwelming.

De oogreflex bleek een trage klinische indicator voor het bepalen van bewusteloosheid.

Aanbevelingen

Het verdient aanbeveling om fixeren, verdoven en aan snijden op een dragende restrainer met aan het einde een kopfixatietoestel te laten plaats vinden bij runderen om de stress tijdens het draaien te voorkomen.
Uit de literatuur en het huidige onderzoek blijkt dat bewustzijn bij runderen na alleen een halssnede gemiddeld ongeveer 1,5 minuut aanhoudt en kan oplopen tot 4 minuten. Gedurende deze periode kan het dier mogelijk pijn ervaren Er wordt aanbevolen mechanisch of elektrisch te bedwelmen voor of direct na de halssnede.

Summary

Introduction

According to the EU Council Directive on the protection of animals, 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. Placement in the restrainer is potentially very stressful and requires special attendance by the operative. Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to approved gas mixtures. The Directive makes an exception to stunning for ritual slaughter. Some Halal groups accept captive bolt stunning after the cut and/or electrical stunning. Where animals are killed without prior stunning checks shall be carried out to ensure that the animals do not present any signs of consciousness or sensibility before being released from restraint.
The aim of this study is to assess brain and heart activity during restraining and rotating the animal 90^{o}, 120^{o} or 180^{o} and before and after neck cutting, neck cutting followed by captive bold and electrical stunning before neck cutting. In addition, blood values related to energy metabolism were measured and analyzed.

Materials and methods

Thirty-one pink veal calves of different breeds were delivered at the slaughterhouse from different commercial farms. Before the experiment the animals were placed in specially separated lairage to sample blood and equip them with EEG and ECG electrodes. Afterwards they were driven to the restrainer, blood sampled once again, fixed head restrained, rotated to 90^{o}, 120^{o} or 180^{o} and were either immediately neck cut, neck cut and captive bolt stunned or stunned electrically and then neck cut.

Results

After neck cutting the CD score fell slowly as did the %power of the higher frequencies. The corneal reflex response ceased 135 ± 57 s after the neck cut. A correlation coefficient of $R = 0.6$ ($P < 0.01$) was determined between CD values and %power of beta waves. The CD scores and the %power of beta waves decreased sharply after captive bolt stunning. A correlation coefficient of $R = 0.8$ ($P < 0.01$) was determined between CD scores and beta wave %power. After electrical stunning a general epileptiform insult was observed and characterized with EEG by tonic, clonic and exhaustion phases. Both CD scores and beta wave %power decreased sharply, delta waves increased after electrical stunning. $R = 0.04$ ($P < 0.01$)
The heartbeat rate in lairage was 84 - 92 beats/min and increased significantly ($p<0.05$) to 113 - 118 at the entrance of the restrainer and further on to 126 – 150 beats/min when rotated at 90^{o}, 120^{o} and 180^{o}. Heartbeat variability also decreased significantly ($P<0.05$).
Interesting increases were observed in blood oxygen values between lairage and entrance to the restrainer and in BE values after rotation to 180^{o}.

Conclusions

It can be concluded that rotation of the restrainer compromised pink veal calf since the heartbeat rate increased and variability in heartbeat rate fell after rotation. A difference between rotation of 90^{o}, 120^{o} and 180^{o} was not observed.

Unconsciousness could be achieved
on average approximately 80 s after the neck cut,
4 s after the neck cut by captive bolt stunning
before the neck cut after electrical stunning.

The corneal reflex appeared to be a very conservative clinical parameter for measuring the state of consciousness.

Recommendations

A double rail conveyor restrainer for cattle was developed, which makes it possible to cut the neck in an upright position using head restraining. It is recommended to use such a restrainer, because rotating increased the stress.

It is reported in literature and observed in our study that unconsciousness in cattle occurred on average 1.5 minute after the neck cut and may remain up to 4 minutes during which pain sensation may be present. It is recommended to stun mechanically or electrically before or just after neck cutting.

Table of contents

1 Introduction

According to the EU Council Directive (1993) and future Council Regulation (2009) on the protection of animals, 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. Placement in the restrainer may cause a lot of stress and require additional attention from the operator. Fear is a major cause for stress in cattle due to the new environment and the fixation of the body. (Grandin, 1997; von Wenzlawowicz and von Holleben, 2007). The degree of fear is related to the breed, sex, age and experience of the animal. The animal can be calmed by the operators and other companion animals (Grandin, 1997; von Wenzlawowicz and von Holleben, 2007). Cattle can be neck cut in the restrainer in a standing position or rotated in e.g. a Weinberg apparatus. Blood cortical values increased significantly and O_2 saturation decreased significantly after rotation to 180^o (Dunn, 1990; Tagawa et al., 1994). The Federation of Veterinarians in Europe (FVE) proposed the prohibition of the 180^o rotation (FVE, 2005). Another detrimental aspect they suggest is the pressure that the intestines can place on heart and lungs causing additional stress.

Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to approved gas mixtures. The Directive makes an exception to stunning for ritual slaughter Some Halal groups do not accept any form of stunning whereas others do accept captive bolt stunning after the cut or electrical stunning before the cut. The animal needs to be intact before slaughter and captive bolt or electrical stunning may affect the integrity of the animal.

In captive bolt stunning methods the important aim is to cause tissue damage by transmitting the energy from the missile to the brain. In general, penetration of a missile into the brain can cause injury in one of three ways, depending on its velocity and shape: by laceration and crushing (< 100 m/s), by shock waves (about 100 to 300 m/s) and by temporary cavitation (> 300 m/s) (Hopkinson & Marshall, 1956).
Missiles used for stunning and killing of animals are a bullet, a bolt, water jet and air pressure. Immediately after penetration the animals express a tonic spasm for approximately 10 s prior to relaxation, however, excessive convulsions may occur (Eichbaum et al., 1975). When immediately after shooting major changes (delta and theta waves tending towards an iso-electric line) are seen on the EEG (electro encephalogram) it is assumed that the animal is unconscious by analogy to similar EEG changes described in humans (Lambooy, 1982; Lopez da Silva, 1983; EFSA, 2004). Also immediately after captive bolt stunning veal calves express a tonic spasm for approximately 10 s prior to relaxation, however, excessive convulsions often follow (Lambooy & Spanjaard, 1981).

Electrical stunning is a commonly used method for farmed species, however use is limited for cattle. It involves electrical stimulation of the brain by placing electrodes on either side of the head, or on the head and body so that current passes through the brain and heart. It is important that an adequate voltage is used to drive sufficient current through the animal. This stimulation of the brain causes the equivalent of a generalized epileptiform brain activity accompanied by seizures indicative of unconsciousness and insensibility (Lambooij, 2004). 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 and calves (Lambooy, 1982; Anil, 1991, Anil and McKinstry, 1992). The eye-reflex can not be used, because the reflex is blocked during the tonic phase and may occur spontaneously during the clonic phase (Roos & Koopmans, 1936). During epilepsy the brain remains in a highly stimulated state being unable to respond to stimuli. Another contributing factor is the release of several neurotransmitters in the brain during such an insult. The first phase induced by the stun produces the tonic phase through release of the excitatory neurotransmitter glutamate. This is followed by the release of GABA that assists in the recovery if the animal is not killed. (Cook et al., 1996)

The time period to unconsciousness lasts longer when the neck cut is performed in unstunned animals as compared to animals that are stunned by electric shock or mechanically (penetrating captive bolt). The neck cut has to be performed in two fluent movements, but during Jewish slaughter a mean of 3.2 cuts were needed and with Halal slaughter a mean of 5.2 cuts was reported. Even then the main arteries in the neck were not completely or incorrectly severed in one out of ten animals (Gregory et al., 2008). During regular stunned slaughter there are also concerns about the restraining method prior to mechanical stunning, which is often stressful for the animals (Grandin, 2001).

The aim of this study is to assess brain and heart activity during restraining and rotating the animal 90^o, 120^o or 180^o and before and after neck cutting, neck cutting followed by captive bold and electrical stunning before neck cutting. In addition, blood values related to energy metabolism were measured and analyzed.

2 Materials and methods.

2.1 Animals.

Thirty-one pink veal calves delivered at the slaughterhouse from different commercial farms and mixed breeds (red or black and white) were randomly selected from groups in lairage. It was possible to perform experiments with approximately 4 to 6 animals per day divided over 7 days After slaughter the carcasses were weighed. The warm slaughter weight averaged 185 ± 26 kg.

Photo 1: A pink veal calf was equipped with EEG and ECG electrodes.

2.2 Experimental design

At the start of the experiment the animals were placed and fixed individually to sample blood and equip them with EEG and ECG electrodes. (Photo 1). The rotation and stunning procedures were randomly allocated between animals. After the preparations they were driven to the restrainer, blood sampled once again, fixed-head restrained, rotated and immediately either neck cut or neck cut and captive bolt stunned or stunned electrically and throat cut (Table 1). Blood was sampled during exsanguination. In the two minutes after the cut the corneal reflex was measured every 30 s (except after electrical stunning), there after the calf was expelled from the restrainer, bled out further and slaughtered.

Table 1 Rotating and neck cutting or stunning and neck cutting procedures

Rotation	Neck cutting / stunning	N
	Neck cutting	3
90°	Neck cutting followed by captive bolt	5
	Electrical stunning followed by neck cutting	3
	Neck cutting	4
120°	Neck cutting followed by captive bolt	4
	Electrical stunning followed by neck cutting	3
	Neck cutting	3
180°	Neck cutting followed by captive bolt	3
	Electrical stunning followed by neck cutting	3

Each calf was selected at random from a group, fastened with a rope halter and blood was sampled from the vena in the tail (*V. caudalis media*) (70 µml). Blood was also sampled before entering the restrainer and during exsanguination. Directly after sampling, the blood was analyzed using an ABL80 Flex (Radiometer Medical ApS Brønshøj, Denmark)
The following parameters were analyzed:

pH	acidity or alkalinity of the venous blood
pCO_2	pressure of carbon dioxide in the venous blood
pO_2	pressure of oxygen in the venous blood
sO_2	saturation of oxygen in the venous blood
BE	base excess (indication of acidity / alkalinity in the blood)
Hb	hemoglobin content
Ht	heamatocriet
Glucose	level of glucose in the venous blood

Each calf was equipped with EEG and ECG electrodes covered by a cloth on the head, protective tubing on the electrode leads and an elastic band around the body to hold the logging device in position (Photo 1). The measuring equipment used was specially designed by the Royal Veterinary College in London (Lowe et al, 2007). The measurement equipment was placed in a steel box covered by a leather bag. Before positioning the band and the electrode pads the skin was shaved, cleaned with 70^{o} alcohol and surgical glue applied to secure the electrode pads to the surface of the skin. The EEG electrode pads were placed on the skin of the skull: one electrode 2 cm to the right and one electrode 2 cm to the left of the sagittal suture and 3 cm of the imaginary transverse line at the caudal margin of the eyes. The ECG electrodes were placed caudal the olecranon on both sides of the brest. The earth electrode for both EEG and ECG was placed on the breast dorsally to the left electrode. Recording started immediately after the equipment was installed and ended after the animal was expelled from the restrainer. Both EEG and ECG were recorded from activation of equipment until 2 minutes after neck cutting. The EEG and ECG recordings were later analyzed for changes in the waveforms, frequency and suppression.
The electrical activity recorded on the EEG can be classified into delta (0 -4 Hz), theta (4-7 Hz), alpha (8-13 Hz) and beta (> 13 Hz) frequency bands. In alpha and beta rhythms, the animal is considered conscious (Kooi et al., 1978).
The ECG was analyzed for heart rate in beats per minute and heart rate variability i.e. the LF/HF ratio. This is the ratio of the absolute powers in the low frequency and high frequency bands (American Heart Association, 2009).

When entered in the restrainer the head was restrained and the calf rotated to either 90^{o}, 120^{o} or 180^{o} (Table 1).Thereafter the animal was stunned by captive bolt (Cash Bulldozer .25; Accles & Shelvoke, West Midlends, UK), at 300 V during 3 s or just neck cut. The power supply (Stork RMS, Lichtenvoorde, Netherlands) delivered a constant voltage (50 Hz a.c., sinusoidal). After stunning the calves were neck cut as soon as possible while restrained.

2.3 Ethics

The experiment was approved beforehand by the Ethical Committee of the Animal Sciences Group of Wageningen UR.

2.4 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 customized to measure depth of anesthesia 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 a reduction in CD to 60% of the baseline value was an indicator of unconsciousness (Broek, 2003).

The EEG signals were computed by FFT (Fast Fourier Transform) using Hanning type (LabChart7 Pro. V7.1.2, AD Instruments, Cologne, Germany). The power spectrum was calculated by means of 512 points with an input averaging factor of 5 and a power smoothing factor of 32. An FFT was computed before and after neck cutting. The frequencies of the waves recorded on the EEG were divided into 4 subgroups: theta waves 1-4 Hz, delta waves 4-8 Hz, alpha waves 8-13 Hz and beta waves 13-32 Hz (Kooi et al., 1978).
An analysis of variance was used to analyze the heart rate and blood parameters (Genstat, 2008). A correlation (R) between the CD and %power of the beta waves was analyzed including a T-test.

3 Results.

3.1 Brain activity

Rotation of the animals in the restrainer did not affect CD analyses scores or EEG analyses.

Neck cutting
At the beginning of the EEG recording in the lairage the pink veal calves showed a high CD score and a high %power of beta waves. After neck cutting there was a slow decrease in both values (Figure 1a and 1b). The correlation was R = 0.6 (P< 0.01) between CD values and the %power of beta waves. An iso-electric line on the EEG was observed 42 ± 7 s after the neck cut, whereas the corneal reflex response ceased after 135 ± 57 s.

Neck cutting followed by captive bolt stunning
The CD scores and the %power of beta waves varied during waiting and driving to the stunner. Both values decreased sharply after captive bolt stunning and the %power of delta and theta waves increased sharply (Table 2a and 2b). The correlation was R = 0.8 (P < 0.01) between CD scores and beta waves %power. An iso-electric line was observed 7 ± 2 s post neck cutting and the corneal reflex was disappeared after the first trial (30 s after the neck cut).

Electrical stunning before neck cutting
After electrical stunning a general epileptiform insult was observed EEG characterized by tonic, clonic and exhaustion phases. Both CD scores and beta wave %power decreased sharply, whereas delta waves increased after electrical stunning. However this decrease was followed by an opposite increase and decrease, respectively. Thereafter a slow decrease and increase was observed, respectively (Table 3a and 3b). The correlation was R = 0.4 (P < 0.01) between CD scores and beta waves %power.

Figure 1a Correlation dimension analyses (average ± SD) of pink veal calves before and after neck cutting. It is suggested that a reduction in score to 60% of the baseline value was an indicator of unconsciousness (Broek, 2003).

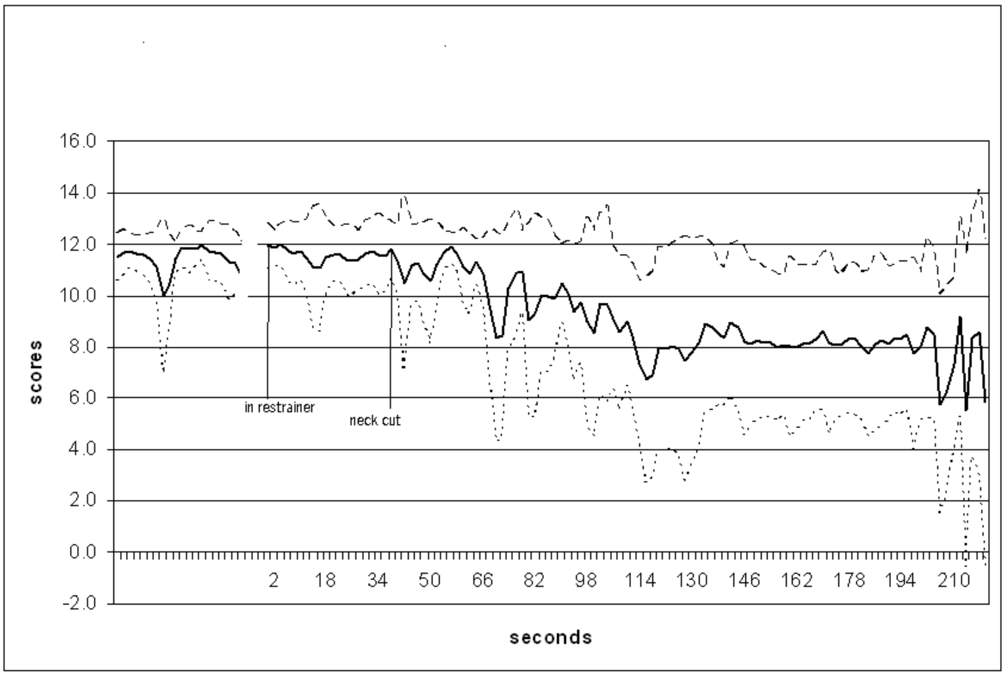


Figure 1b %Power of the different EEG frequency groups of pink veal calves just before and after neck cutting. In alpha (8 – 13 Hz) and beta (13 – 32 Hz) rhythms, the animal is considered conscious (Kooi et al., 1978).

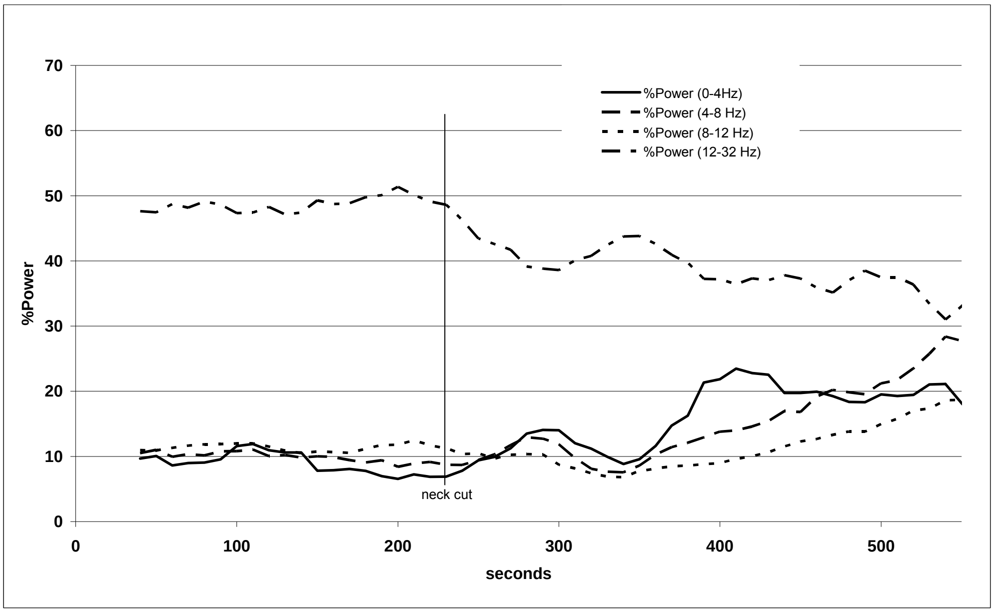


Figure 2a Correlation dimension analyses (average ± SD) of pink veal calves before and after neck cutting followed by captive bolt stunning It is suggested that a reduction in score to 60% of the baseline value was an indicator of unconsciousness (Broek, 2003).

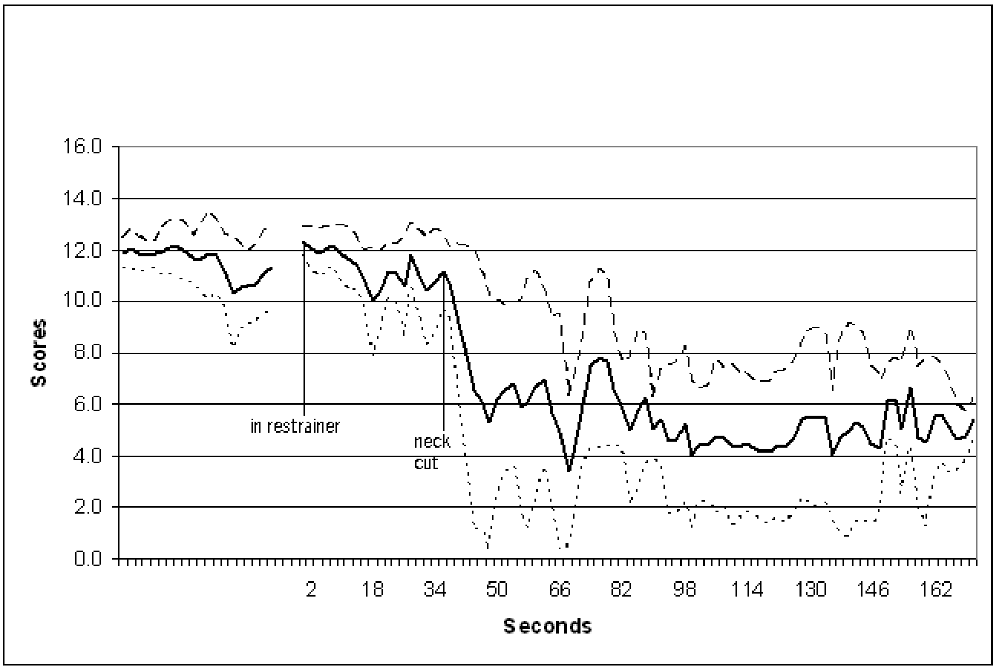


Figure 2b %Power of the different EEG frequency groups of pink veal calves before and after neck cutting followed by captive bolt stunning. In alpha (8 – 13 Hz) and beta (13 – 32 Hz) rhythms, the animal is considered conscious (Kooi et al., 1978).

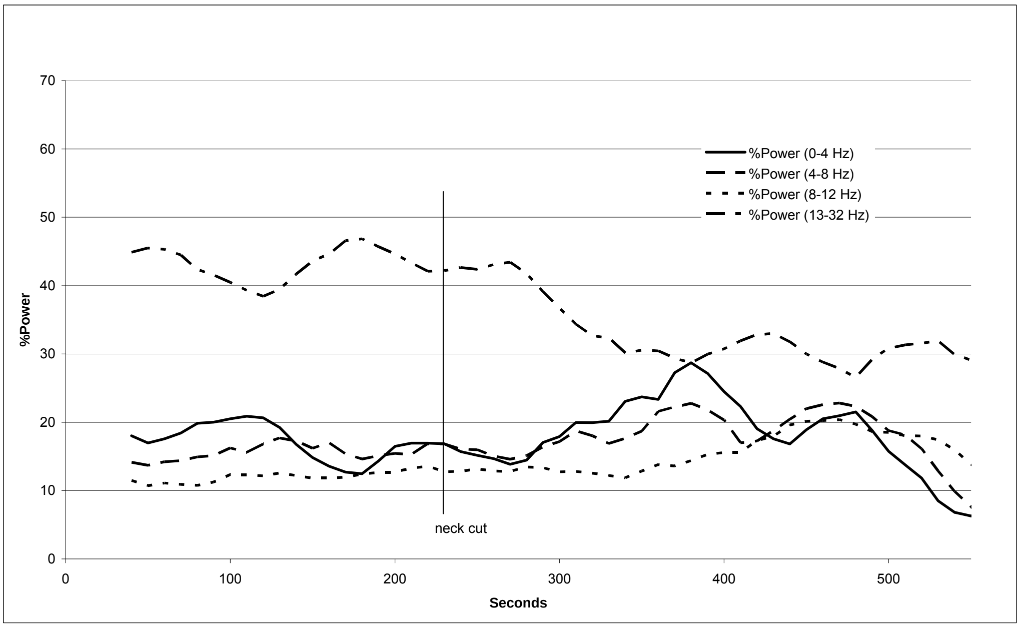


Figure 3a Correlation dimension analyses (average ± SD) of pink veal calves before and after electrical stunning and neck cutting. It is suggested that a reduction in score to 60% of the baseline value was an indicator of unconsciousness (Broek, 2003).

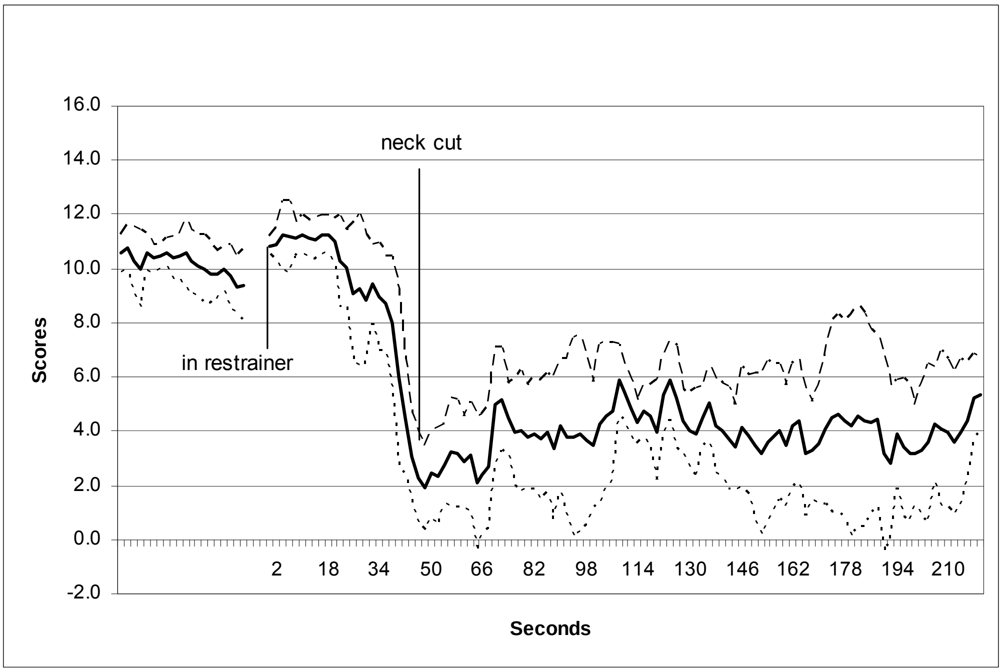
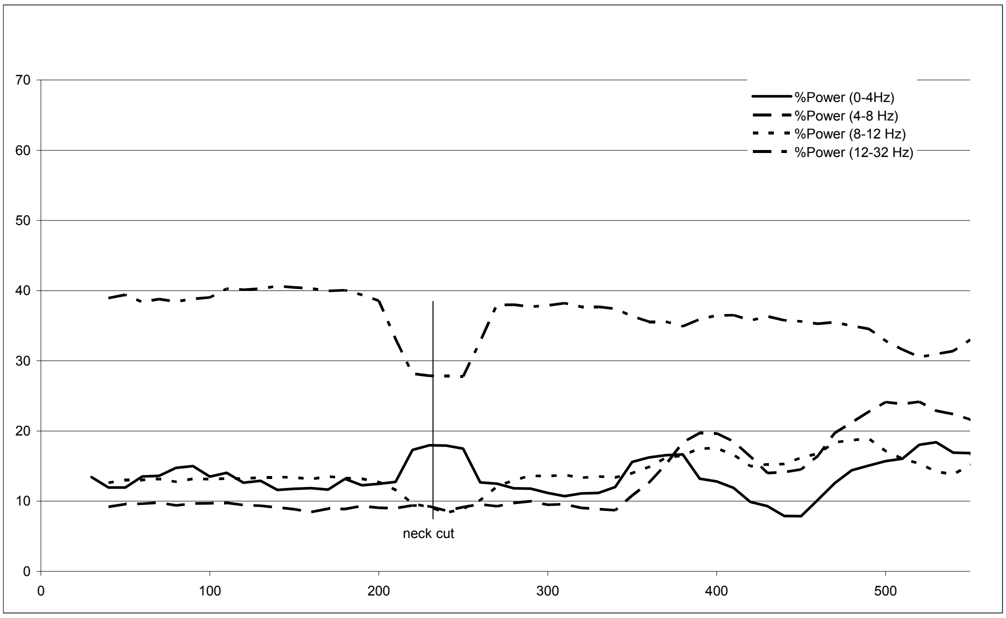


Figure 3b %Power of the different EEG frequency groups of pink veal calves before and after electrical stunning followed by neck cutting. In alpha (8 – 13 Hz) and beta (13 – 32 Hz) rhythms, the animal is considered conscious (Kooi et al., 1978).



3.2 Heart activity

The heart rate during lairage was recorded at 84 - 92 beats/min and increased significantly ($p<0.05$) to 113 - 118 beats/min during driving to the restrainer (Table 2a, b). The heart rate increased further on significantly ($p<0.05$) to 126 - 138 beats/min when rotated to 90°, 120° and 180°. After rotation heart variability (LF/HF ratio) also changed significantly ($P<0.05$) (Table 2a, b).

The heart rate increased further when the animals were neck cut or neck cut combined with stunning after or before the cut. Due to the electrical stunning the heart rate increased to a high level (Table 3).

Table 2a Heart rate in beats/min and heart variability (LF/HF ratio) after the start of recording, at the entrance to the restrainer and after rotation of pink veal calves

Rotation		Start	Entrance	Rotating
90°	Heart rate beats/min	92	113	126
	LF/HF ratio	5.1 ± 5.7	9.3 ± 12.0	0.6 ± 0.4
120°	Heart rate beats/min	84	115	150
	LF/HF ratio	5.4 ± 9.0	27.3 ± 46.3	0.6 ± 0.7
180°	Heart rate beats/min	85	118	138
	LF/HF ratio	2.9 ± 3.2	7.8 ± 9.1	0.5 ± 0.3

Table 2b Statistical differences (P value) in heart rate in beats/min and heart variability (LF/HF ratio) at the beginning of recording, at the entrance to the restrainer and after rotation of pink veal calves. (See Table 2a).

Rotation		Start/entrance	Entrance/rotating
90°	Heart rate beats/min	0.02	0.08
	LF/HF ratio	0.35	0.06
120°	Heart rate beats/min	0.00	0.01
	LF/HF ratio	0.26	0.18
180°	Heart rate beats/min	0.00	0.04
	LF/HF ratio	0.14	0.03

Table 3 Heart rate in beats/min (mean ± SD) just after neck cutting.

	Seconds after cutting		
procedure	just	30 s	60 s
Neck cutting	166 ± 16	173 ± 61	202 ± 51
Captive bolt stunning after neck cutting	173± 31	163 ± 47	140 ± 23
Electrical stunning before neck cutting	239 ± 57	238	282 ± 76

3.3 Blood parameters

Differences in blood parameters were not observed between stunning or non- stunning methods. Significant differences were observed between the beginning, entrance to the restrainer and rotating and between the degrees of rotation (Table 4a, b). Noticeable increase was observed in oxygen value from the beginning and during entrance to the restrainer and in BE values after rotation to 180°.

Table 4a Value of blood parameters after the starting recording, at the entrance to the restrainer and after rotation of pink veal calves.

Parameter	Start	Entrance	Rotating	SE	P value
pH	7.45	7.48	7.46	0.02	0.13
pCO_2 kPa	5.49	5.23	5.67	0.30	0.55
pO_2 kPa	2.21	2.46	2.09	0.13	0.01
sO_2 %	89.3	92.7	84.9	3.2	0.02
BE mmol/l	4.06	4.76	4.91	0.39	0.16
Hb mmol/l	1.86	1.84	1.93	0.04	0.08
Ht %	34.5	34.5	35.2	0.4	0.08
Glucose mmol/l	6.20	6.09	6.11	0.17	0.91

Table 4b Value of blood parameters after beginning of recording, and after rotation of pink veal calves to 90°, 120° and 180°.

Parameter	90°	120°	180°	SE	P value
pH	7.44	7.48	7.46	0.03	0.02*
pCO_2 kPa	5.81	4.94	5.63	0.55	0.05
pO_2 kPa	2.31	2.38	2.07	0.16	0.22
sO_2 %	89.4	93.0	84.5	3.5	0.06
BE mmol/l	3.98	3.93	5.81	0.87	0.02*
Hb mmol/l	1.83	1.91	1.87	0.06	0.38
Ht %	34.4	35.2	34.8	0.6	0.36
Glucose mmol/l	6.14	5.93	6.31	0.44	0.66

* Interaction driving to restrainer/rotation

4 Discussion.

Humane slaughter regulations aim to minimize anxiety, pain, distress or suffering at slaughter. In mammals, stunning and stun-to-kill methods should induce unequivocal and immediate loss of consciousness and pain sensation and when not immediate non-aversive (EFSA-AHAW/04-027, 2004). The insight into the process of attaining unconsciousness after neck cutting with or without stunning provided by neural and physiological studies is very important. Assessment of neural and physiological parameters may elucidate the humaneness of the stunning and killing procedure. EEG, rhythm analyses (FFT, CD) and neurotransmitter release measurements have been used to assess the effectiveness of mechanical and electrical head-only stun duration on welfare and the effect on pain sensation and suffering immediately after neck cutting. CD analysis is a relatively new technique that has been customized to measure depth of anesthesia in humans (Broek, van den 2003). The small amplitude, high frequency (conscious) 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. Studies with chickens suggest 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).

According to the CD scores there is a slow decrease after neck-cutting and an immediately large decrease after captive bolt and electrical stunning (Figures 1, 2 and 3) which indicate a slow induction of unconsciousness after neck cutting and a possible pain sensation during neck cutting. Since CD score analyses have not yet been validated for cattle frequency power analyses were performed, which show the same characteristics. Quantitative analyses of components of the EEG are used to assess painful procedures due to noxious sensory inputs. FFT is a mathematical process that changes the raw EEG signal from the time domain to the frequency domain, generating a power spectrum (Murrell & Johnson, 2006; Mellor et al., 2009). It is stated that consciousness is awareness of the world around and of the own body, where unconsciousness means unarguable unresponsiveness. In a state of unconsciousness the EEG is always abnormal (Lopes da Silva, 1984). Appearance of theta and delta waves and spikes towards an iso-electric line (minimal brain activity) indicate cessation of brain activity (Lopes da Silva 1983). Since theta and delta waves tending toward no brain activity occurred after captive bolt stunning and a general epileptiform insult occurred after electrical head only stunning in veal calves consciousness and the possibility of perception of pain could be excluded (Lambooij & Spanjaard, 1981, 1982). In our study we observed a slow decrease and increase in higher and lower frequencies on the EEG, respectively after neck cutting (Figures 1a, 1b)) and an immediate change after captive bolt stunning (Figures 2a, 2b) and after electrical stunning (Figures 3a, 3b). The results of a minimal anesthesia model in calves showed that the impact of ventral-neck incision was associated with significant noxious sensory input that would have been likely to be perceived as pain in conscious animals (Gibson et al., 2009a; Mellor et al., 2009). When applying a non-penetrative captive bolt stun 5 seconds after the ventral-neck incision ameliorated the noxious sensory input caused by the incision (Gibson et al., 2009d), and showed that the stun prevented the subsequent development of responses in the EEG to noxious sensory input in most of the animals (Mellor et al., 2009). These results support our findings using CD scores.

In Figure 1a the 60% score (score 8) and in Figure 1b a decrease in alpha / beta rhythms in the EEG or unconsciousness was reached after approximately 80 s and an iso-electric line approximately 42 s after the neck cut, whereas the corneal reflex response ceased after 135 ± 57 s. This indicates that this clinical parameter is very conservative. Cattle have a basi-occipito plexus that allow blood to pass via an alternative routing (Baldwin, 1960), moreover they are prone to develop aneurisms (Gregory at al., 2006). This combination presents a risk of prolonged periods of consciousness following neck cutting. A study in an abattoir (Gregory et al., 2009) showed that 90% of cattle collapsed in 34 s after the neck cut and 8% took longer than 60 s which is in the same order of the 50%power found by Gibson (2009a) and the occurrence of the iso-electric line in our study. Seventy-one percent of the cattle that took more than 75 s (up to 4 minutes) to collapse had ballooning or false aneurysms in the cardiac ends of the carotid arteries (Gregory et al., 2009). In our study ballooning or false aneurysms might play a role in the prolonged duration of occurrence of unconsciousness. During exsanguination after electrical stunning there was an increase in the CD score and power% at higher frequencies. Once again this may be due to ballooning or false aneurisms. The same phenomenon was observed in salmon showing that one out of 3 fish recovered during exsanguination after electrical stunning. (Lambooij et al 2009)

A restraining device which is generally used in Europe is the Weinberg casting pen which consists of a narrow stall that slowly inverts the animal until it is lying on its back (Dunn, 1990; Grandin, 2005)
For our experiments this restrainer was adapted to also rotate to a 90^o and 120^o position. The head of the calves were restrained before they were rotated in our experiments since it was found that there was more vigorous struggling when head was restrained after inversion (Gregory, 2005). Animals in this casting pen had much higher vocalization and cortisol levels compared to cattle restrained in an upright position and blood O_2 saturation decreased significantly after rotation for 180^o (Dunn, 1990; Tagawa et al., 1994; Grandin, 2005). We found significant effects on blood parameters, decreasing oxygen level after increased angle of rotation and also an effect on BE after rotation to 180^o. However, interaction with driving to the restrainer compounded these latter observations. The effects could be explained by the pressing of the rumen on the diaphragm and thoracic organs (Farm Animal Welfare Council, 1985). The heart rate variability reduced significantly after rotation. Reduced heart rate variability is characterized by signs of sympathetic activation such as faster heart rates and high levels of catecholamine’s (Malik, 1996). Neck cutting, however, is easier to perform in cattle in a rotated position but it leads to increased stress and an un-natural position when turned dorsally. Turning to positions between upright and lateral recumbency (e.g. 45^o or 90^o) has the potential to decrease stress (Velarde et al., 2010). A double rail conveyor restrainer for cattle or sheep in an upright position with the legs straddling and the body supported by the belly has been developed, however never applied in practice. The animals experienced less stress in this system (Giger et al., 1977). Later a similar system was constructed for cattle for stunning by captive bolt which can also be used for non-stunning (Grandin, 1986, 1989). Recently, a double rail was also developed for single cattle and sheep in small slaughter houses (Regenstein, pers. comm.).

5 Conclusions and recommendation

Conclusions

It can be concluded that rotation of the restrainer compromised pink veal calf since the heartbeat rate increased and variability in heartbeat rate fell after rotation. A difference between rotation of 90^o, 120^o and 180^o was not observed.

Unconsciousness could be achieved
- on average approximately 80 s after the neck cut,
- 4 s after the neck cut by captive bolt stunning
- before the neck cut after electrical stunning.

The corneal reflex appeared to be a very conservative clinical parameter for measuring the state of consciousness.

Recommendations

A double rail conveyor restrainer for cattle was developed, which makes it possible to cut the neck in an upright position using head restraining. It is recommended to use such a restrainer, because rotating increased the stress.

It is reported in literature and observed in our study that unconsciousness in cattle occurred on average 1.5 minute after the neck cut and may remain up to 4 minutes during which pain sensation may be present. It is recommended to stun mechanically or electrically before or just after neck cutting

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References

Anil, M.H., 1991. Studies on the return of physical reflexes in pigs following electrical stunning. Meat Science 30, 13-21.
Anil, M.H., and McKinstry, J.L., 1992. The effectiveness of high frequency electrical stunning of pigs. Meat Science 31: 481-491.
Baldwin, B. A.; Bell, F. R., 1963. Blood flow in the carotid and vertebral arteries of the sheep and calf. Journal Physiology 167, 448-462
Broek, P.L.C. van den, 2003. Monitoring anaesthetic depth. Modification, evaluation and application of the correlation dimension. PhD Thesis Nijmegen University Press The Netherlands.
Broek, P.L.C., van den, van Egmond, J., van Rijn, C.M., Takens, F., Coenen, A.M.L. and Booij, L.H.D.J., 2005. Feasibility of real-time calculation of correlation integral derived statistics applied to EEG time series. Physica D 203, 198-208.
Coenen, A.M.L. and van den Broek. P.L.C. 2005. Correlation dimension of the chicken EEG during sleeping, waking and drowsiness. Nederlandse Vereniging voor Slaap-Waak Onderzoek, Heeze Nederland 16, 43- 46.
Cook, C.J., Maasland, S.A., Devine, C.E., Gilbert, K.V., and Blackmore, D.K., 1996. Changes in the release of amino acid neurotransmitters in the brains of calves and sheep after head-only electrical stunning and throat cutting. Research in Veterinary Science 60, 225-261.
Dunn, C.S., 1990. Stress Reactions of Cattle Undergoing Ritual Slaughter Using 2 Methods of Restraint. Veterinary Record 126, 522-525.
Eichbaum, F.W., Slewer, O. and Yasaka, W.J., 1975. Post decapitation convulsions and their inhibition by drugs. Experimental Neurology 49, 802-812.
European Food Safety Authority (EFSA), 2004. AHAW/04-027. Welfare aspects of stunning and killing methods. Scientific Report of the Scientific Panel for Animal Health and Welfare on a request from the Commission related to welfare aspects of animal stunning and killing methods (Question N° EFSA-Q-2003-093). Report AHAW/04-027, 241 pp.
European Commission, 1993. Directive 93/119/EC on the protection of animals at the time of slaughter or killing. European Community Official Journal 340: 21–34.
European Union, 2009. Council Regulation No1099/2009 on the protection of animals at the time of killing. Official Journal of the European Union L303/1-30
Farm Animal Welfare Council (1985): Report on the welfare of livestock when slaughtered by religious methods. 262, 0 11 242729 4, Farm Animal Council, Government Buildings, Hook Rise South, Tolworth Surbiton, Surrey, KT6 7NF, 15 pp.
FVE, 2005. FVE-Position Paper; (Federation of Veterinarians of Europe); Slaughter of animals without prior stunning. In: Erna-Graff-Stiftung für Tierschutz, 2005, pp 103-106.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Hulls, C.M., Mitchinson, S.L., Stafford, K.J., Johnstone, A, C, and Mellor, D.J., 2009a. Electroencephalographic responses of halothane anaesthetised calves to slaughter by ventral-neck incision without prior stunning. New Zealand Veterinary Journal 57, 77–83.
Gibson, T.J., Johnson, C.B., Murrell, J.C., Mitchinson S.L., Stafford, K.J., Mellor, D.J., 2009b. Amelioration of electroencephalographic responses to slaughter by nonpenetrative captive-bolt stunning after ventral-neck incision in halothane anaesthetised calves. New Zealand Veterinary Journal 57, 96–101.
Giger, W., Prince, R.P., Westervelt, R.G., Kinsman, D.M., 1977. Experiment for low-stress, small animal slaughter. Transactions of the ASAE 20, 571-578.
Grandin, T., 1986. New design for a restrainer. Meat and Poultry. December P 61.
Grandin, T. 1989. Calmer – because they are carried. Beef, October. Webb Agricultural Publishers.
Grandin, T., 1997. Assessment of stress during handling and transport. *J. Anim. Sci*. 75, 249-257.
Grandin, T., 2001. Welfare of cattle during slaughter and the prevention of nonambulatory (downer) cattle. Journal American Veterinary Medical Association 219, 1377-1382.
Grandin, T. 2005. Restraint methods for holding animals during ritual slaughter. In: Luy J. et al. [Ed.] Animal Welfare at Ritual Slaughter. DVG Service gmbH, http://www.erna-graff-stiftung.de/cms/download/tierschutz_bei_der_rituellen_schlachtung.pdf, 64- 6.
Gregory, N. G., 2005. Recent concerns about stunning and slaughter. Meat Science 70, 481-49.
Gregory, N. G.; Shaw, F. D.; Whitford, J. C.; Patterson-Kane, J. C., 2006. Prevalence of ballooning of the severed carotid arteries at slaughter in cattle, calves and sheep. Meat Science 74, 655-657.
Gregory, N. G.; Wenzlawowiz, M. v.; Alam, R. M.; Anil, H.; Yesildere, T.; Silva-Fletcher, A., 2008. False aneurysms in carotid arteries of cattle and water buffalo during shechita and halal slaughter. Meat Science 79, 285-288.

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Gregory, N. G.; Fielding, H. R.; Wenzlawowicz, M. v.; Holleben, K. v., 2010. Time to collapse following slaughter without stunning in cattle. Meat Science 85, 66-69.
Hopkinson, D.A.W. and Marshall, T.K., 1967. Firearm injuries. British Journal for Surgery 54, 344-353.
Kooi, K.A., Tucker, R.P., Marshal, R.R., 1978. Fundamentals of electro encephalography. Second ed. Harper and Row, New York, pp 125 – 145.
Lambooy, E. 1982.Electrical stunning of sheep. Meat Science, 6:123-135.
Lambooij, E., 2004. Electrical stunning. Encyclopedia of Meat Sciences (Eds. W.K. Jensen, C. Devine and M Dikeman. Elsevier, Oxford. ISBN 0-12-464970. Pp 1342-1348.
Lambooy, E. and Spanjaard, W.J., 1981. Effect of the shooting position on the stunning of veal calves by captive bolt. The Veterinary Record, 109 16 359-361.
Lambooy, E, & W.J. Spanjaard, 1982. Electrical stunning of veal calves. *Meat Science* 6, 15-25.
Lambooij, E., Grimsbø, E., van de Vis, J.W., Reimert, H.G.M., Nortvedt, R., Roth, B., 2010. Percussion and electrical stunning of Atlantic salmon (*Salmo salar*) after dewatering and subsequent effect on brain and heart activities. Aquaculture 300, 107-112.
Lopes da Silva, H.F, 1983. The assessment of consciousness: General principles and practical aspects. In: Stunning of animals for slaughter (G. Eikelenboom, Ed.), Martinus Nijhoff. The Hague, pp 3-12.
Lowe, J.C., S.M. Abeyesinghe, S.M., Demmers, T.G.M., Wathes, C.M., and McKeegan, D.E.F. 2007. A novel telemetric logging system for recording physiological signals in unrestrained animals. *Computers* and Electronics in Agriculture, 57, p.74-79.
Malik, M., 1996. Heart rate variability; Standards of measurement, physiological interpretation and clinical use. Circulation 93, 1043-1065.
McKeegan, D.E.F., McIntyre, J.A., Demmers, T.G.M., Lowe, J.C., Wathes, C.M., van den Broek, P.L.C., Coenen, A.M.L., and Gentle, M.J., 2007. Physiological and behavioural responses of broilers to controlled atmosphere stunning: implications for welfare. Animal welfare, 16:409-426.
Mellor, D. J.; Gibson, T. J.; Johnson, C. B., 2009. A re-evaluation of the need to stun calves prior to slaughter by ventral-neck incision: An introductory review. New Zealand Veterinary Journal 57, 74-76.
Murrell JC and Johnson CB., 2006 Neurophysiological techniques to assess pain in animals. Journal of Veterinary Pharmacology and Therapeutics 29, 325–35.
Regenstein, J.M. Personnel communication Professor of Food Science. Cornell University Ithaca, NY 14853-7201.
Roos, J. and Koopmans, S., 1936. The value of the eye-reflex in animals submitted to the so-called electrical stunning. Veterinary Journal 92, 127-137.
Tagawa, M., Okano, S., Sako, T., Orima, H., Steffey, E.P., 1994. Effect of Change in Body Position on Cardiopulmonary Function and Plasma-Cortisol in Cattle. Journal Veterinary Medical Science 56, 131-134.
Velarde, A., Rodriguez P., Fuentes, C., Llonch, P., Holleben, K. von, Wenzlawowicz, M. von, Anil, H., Miele, M., Cenci Goga, B., Lambooij B., Zivotofsky A., Gregory, N., Bergeaud-Blackler F., Dalmaa, A., 2010. Main outcomes and recommendations to good animal welfare practices during religious slaughter. http://www.dialrel.eu/
Wenzlawowicz, M. von, von Holleben, K., 2007. Tierschutz bei der betäubungslosen Schlachtung aus religiösen Gründen [Animal welfare at religious slaughter without stunning], publication of an expertise on behalf of the Community of the German Veterinary Chambers and Veterinary Associations - review article. Deutsches Tierärzteblatt 55, 1374-1386; http://www.bundestieraerztekammer.de /datei.htm?filename=gutachten_schaechten.pdf&themen_id=4882.