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SCIENTIFIC OPINION

Scientific Opinion on the scientific assessment of studies on electrical parameters for stunning of small ruminants (ovine and caprine species)[1]

EFSA Panel on Animal Health and Welfare (AHAW)[2,3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

The EFSA Panel on Animal Health and Welfare (AHAW Panel) was asked by the European Commission to deliver a scientific opinion on three studies evaluating electrical parameters for the stunning of lambs and kid goats. The Commission received the studies from the Spanish authorities. One study was in the form of a manuscript entitled 'Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats'. The second study consisted of a summary in English entitled 'Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the carcass quality in *Pascuales* lambs', and the third study consisted of a summary in English entitled 'Effect of electrical stunning with inferior intensities to 1 Ampere on the induction to the unconsciousness in lambs'. Reported outcomes for different animal categories were inconsistent and contradictory, indicating a need to validate if the stunning equipment delivered the intended current levels to the animals. The head-only stunning and slaughter intervention failed to achieve and/or maintain unconsciousness during bleeding and the head-to-body stunning and slaughter intervention failed to achieve cardiac ventricular fibrillation in all of the animals, as evidenced by the presence of corneal reflex and rhythmic breathing. No evidence of absence of pain and suffering is presented in the studies. The submitted studies are not considered adequate for a full welfare assessment of the alternative stunning method because they do not fulfil the eligibility criteria and the reporting quality criteria defined in the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions or the EFSA opinion on monitoring welfare at slaughter of small ruminants.

KEY WORDS

lambs, goats, low current, electrical, stunning, welfare

[1] On request from the European Commission, Question No EFSA-Q-2014-00109, adopted on 4 February 2015.

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SUMMARY

Following a request from the European Commission, the EFSA Panel on Animal Health and Welfare (AHAW Panel) was asked to deliver a scientific opinion on three studies evaluating electrical parameters for the stunning of lambs and kid goats.

The Commission received the studies from the Spanish authorities. One study was in the form of a manuscript entitled 'Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats'. The second study consisted of a summary in English entitled 'Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the carcass quality in *Pascuales* lambs', and the third study consisted of a summary in English entitled 'Effect of electrical stunning with inferior intensities to 1 Ampere on the induction to the unconsciousness in lambs'.

The assessment focuses on the first term of reference (ToR 1) limiting the scope of this request to the head-only (HO) and head-to-body (HB) electrical stunning of small ruminants (ovine and caprine species). A review of the study was carried out to assess if it provides sufficient scientific detail to evaluate the stunning procedure applied and its welfare outcome (ToR 2). Each of the three working group experts independently reviewed if the eligibility criteria set out in the guidance for electrical interventions (EFSA AHAW Panel, 2013a) were met by the study. If the eligibility criteria were met, the assessment would proceed to ToRs 3 and 4.

The submitted studies were not adequate for a full welfare assessment of the alternative method studied because they do not fulfil the eligibility criteria and the reporting quality criteria defined in the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions (EFSA AHAW Panel, 2013a) or the EFSA opinion on monitoring welfare at slaughter of small ruminants (EFSA AHAW Panel, 2013b). The shortcomings of the studies are identified below.

The reported results indicated inconsistencies of observed outcomes for different animal categories, indicating a need to verify that the stunning equipment delivered the intended current levels. Such validation measurements were not provided. The results of electroencephalography (EEG) are inconsistent and contradictory. The HO electrical stunning and slaughter intervention failed to achieve immediate loss of consciousness and/or maintain unconsciousness during bleeding, and the HB electrical stunning and slaughter intervention failed to achieve immediate loss of consciousness and cardiac ventricular fibrillation in all the animals, as evidenced by the concurrent presence of corneal reflex and rhythmic breathing. No evidence of absence of pain and suffering is presented in the study.

The stunning parameters proposed in the study do not fulfil the animal welfare requirement, which is the successful induction of unconsciousness until the onset of death via slaughter or cardiac ventricular fibrillation.

Following the review of the information provided and evaluation of the stunning procedure applied and its welfare outcome (ToR 1 and ToR 2), it was concluded that the submitted study does not provide enough scientific information upon which to base an assessment of the scientific approach and parameters suggested.

Consequently, a full assessment of the animal welfare implications of the proposed stunning procedure was not undertaken.

The use of live animals in experimental procedures should be minimised as far as possible: specifically the 3R principles (replacement, reduction and refinement) shall be considered in accordance with Directive 2010/63/EU. When a particular parameter aimed at achieving effective stunning and slaughter fails to fulfil the criteria in consecutive animals, the procedure should be terminated.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

Article 4 (2) of Council Regulation (EC) No 1099/2009[4] on the protection of animals at the time of killing allows the Commission to amend stunning parameters laid down in Annex I to this Regulation to take into account scientific and technical progress on the basis of an EFSA opinion. Any such amendments shall ensure a level of animal welfare at least equivalent to that ensured by the existing methods.

At present, a minimum current of 1 Ampere (A) is required for both head-only (Point 4.2 of Chapter II of Annex I to Regulation 1099/2009) and head-to-body (Point 5.1 of Chapter II of Annex I to Regulation 1099/2009) electrical stunning of small ruminants.

Article 2 (f) of Regulation (EC) No 1099/2009 defines 'stunning' as 'any intentionally induced process which causes loss of consciousness and sensibility without pain including any process resulting in instantaneous death'. Furthermore, Article 4 states that 'The loss of consciousness and sensibility should be maintained until the death of the animal'.

Following a previous request, the EFSA adopted an opinion on the stunning of lambs (EFSA AHAW Panel, 2013a) as well as a document on the guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing (EFSA AHAW Panel, 2013a).

The Spanish authorities have sent the Commission new data that they would like to be examined (see attachment) as regards the minimum current for small ruminants for respectively head-only and head-to-body electrical stunning.

In order to reply to this request, the Commission would like to request the EFSA to review the scientific knowledge on the electrical stunning of small ruminants of these studies.

TERMS OF REFERENCE AS PROVIDED BY EUROPEAN COMMISSION

The Commission therefore considers it opportune to request EFSA to prepare a scientific opinion on the use of a lower minimum current than 1 A for electrical stunning of small ruminants.

- The scope of this request is limited to the head-only and head-to-body electrical stunning of small ruminants (ovine and caprine species).
- Review if the study provides sufficient scientific details as to evaluate the stunning procedure applied and its welfare outcome;
- In the case of a favourable reply, carry out a full assessment of the animal welfare implications of the proposed stunning procedure, taking into account other relevant references. In its assessment, EFSA should give its view on the following issues:
  - The extent to which minimum currents lower than 1 A provide a level of animal welfare at least equivalent to that ensured by the use of a minimum current of 1 A;
  - The extent to which the findings of the study are consistent with other sources on electrical stunning of small ruminants (in particular on lowering the current for younger/smaller animals);
  - The extent to which the findings of the study can be valid for different breeds of small ruminants;

[4] Council Regulation (EC) No 1099/2009 of 24 September 2009 on the protection of animals at the time of killing. OJ L 303, 18.11.2009, p. 1–30.

  - Additional requirements possibly linked to the use of minimum currents lower than 1 A for small ruminants, in particular in terms of maximum live weight and possibly of other conditions (minimum voltage, maximum frequency, time of exposure, stun-to-stick interval, etc.).

- Recommend, if necessary, a revision of the electrical requirements applicable for head-only and head-to-body electrical stunning equipment for small ruminants laid down in points 4 and 5 of Chapter II of Annex I to Regulation (EC) No 1099/2009.

ASSESSMENT

1. Introduction

Electrical stunning is widely used for small ruminants and can be performed as head-only (HO) or head-to-body (HB) stunning. Annex I of Council Regulation (EC) No 1099/2009 specifies the minimum currents for HO or HB stunning of sheep and goats, but it does not differentiate between different age groups, e.g. lambs versus adults. Electrical stunning consists of the application of a current to the brain that is sufficiently high to induce grand mal epilepsy, followed by a spreading depression due to hyperpolarisation, rendering the animal unconscious and insensible (EFSA, 2004). According to the Regulation, HO electrical stunning must induce epileptiform activity in the brain and HB electrical stunning must induce epileptiform activity in the brain and cardiac ventricular fibrillation.

A lawful application of new stunning methods in the European Union must ensure a level of welfare at least equivalent to that ensured by the methods already provided in Council Regulation (EC) 1099/2009. The term 'acceptable alternative' is defined as an alternative stunning intervention that is at least as good as those listed in the Council Regulation (EC) 1099/2009. In particular, for interventions that do not induce immediate unconsciousness, the alternative procedure should ensure the absence of pain, distress and suffering until the onset of unconsciousness, and that the animal remains unconscious and insensible until death (EFSA, 2004; EFSA AHAW Panel, 2013a).

In addition, EFSA conducted a stakeholder consultation survey and expert opinion elicitation exercise and produced tool boxes for monitoring welfare at slaughter of food animals, including small ruminants. It is worth stating that, in the opinion on small ruminants, the list of three key indicators of consciousness following ineffective electrical stunning included the presence of rhythmic breathing and corneal reflex (EFSA AHAW Panel, 2013b).

According to Directive 2010/63/EC,[5] new scientific knowledge is available in respect of factors influencing animal welfare, as well as the capacity of animals to sense and express pain, suffering, distress and lasting harm. It is therefore necessary to improve the welfare of animals used in scientific procedures by raising the minimum standards for their protection in line with the latest scientific developments. The choice of methods and the species to be used have a direct impact on both the numbers of animals used and their welfare. The choice of methods should therefore ensure the selection of the method that is able to provide the most satisfactory results and is likely to cause the minimum pain, suffering or distress. The methods selected should use the minimum number of animals that would provide reliable results and require the use of species with the lowest capacity to experience pain, suffering, distress or lasting harm that are optimal for extrapolation into target species.

The Panel on Animal Health and Welfare was asked to deliver a scientific opinion on a compilation of results from three different studies: (1) a study entitled 'Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats' (Llonch, Rodríguez, Casal et al.[6]); (2) a study with an English summary entitled 'Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the carcass quality in *Pascuales* lambs' (Rodríguez, Llonch, Casal et al.[7]); and (3) a study with an English summary entitled 'Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the induction to the unconsciousness in lambs' (Rodríguez, Llonch, Casal et al.[8]). To achieve this, the first step was to define the type of study, critical variables, experimental design, data collection and analysis and reporting needed to supply scientific evidence that a given electrical stunning protocol of lambs and goat kids provides a level of animal welfare at least equivalent to that

[5] Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. OJ L 276, 20.10.2010, p. 33–79.
[6] Report is listed under item 1 in the Section 'Documentation provided to EFSA'.
[7] Report is listed under item 2 in the Section 'Documentation provided to EFSA'.
[8] Report is listed under item 3 in the Section 'Documentation provided to EFSA'.

ensured by the use of a minimum current of 1 A, according to the EFSA guidance document (EFSA AHAW Panel, 2013a). These criteria were applied to the submitted studies.

2. Documentation assessed by EFSA

The Commission received three studies from the Spanish authorities: one study (Llonch, Rodríguez, Casal et al.), in the form of a 29-page manuscript entitled 'Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats'; a second study (Rodríguez, Llonch, Casal et al.) of four pages including a summary in English entitled 'Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the carcass quality in *Pascuales* lambs'; and a third study (Rodríguez, Llonch, Casal et al.) of four pages including a summary in English entitled 'Effect of electrical stunning with inferior intensities to 1 Ampere on the induction to the unconsciousness in lambs'.

3. Assessment approach

The assessment of the submitted studies was carried out in a manner analogous to that described in the guidance document (EFSA AHAW Panel, 2013a). The evaluation was carried out on the study that provided detailed information regarding stunning effectiveness with current levels lower than 1 A in lambs and kid goats (Llonch, Rodríguez, Casal et al.). Two studies (both by Rodríguez, Llonch, Casal et al.) were provided as summaries, with limited experimental details regarding carcass quality and induction of unconsciousness.

The assessment was first conducted independently by each working group (WG) member. The individual assessments were then discussed to reach a consensus on issues over which the experts had expressed different opinions. A detailed evaluation of the reported parameters in the submitted study is presented in Appendix A. The evidence basis for the conclusions drawn is summarised below. Only those aspects that were not considered adequate are commented upon in the text of this opinion.

4. Assessment of eligibility criteria

4.1. Intervention under experimental slaughterhouse conditions

The submitted laboratory study was conducted in an experimental slaughterhouse, equipped with a constant current electrical stunning system supplied with a maximum of 240 volts (Llonch, Rodríguez, Casal et al.). The parameters in the submitted study were assessed (Table 1, Appendix A) when applying a stunning intervention based on electrical stunning for small ruminants, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA guidance (corresponding to Table 3 (EFSA AHAW Panel, 2013a)).

It was noted that, while it was stated in the submitted study that equipment was checked and calibrated daily (Table 1, Appendix A), no details or results of calibrations, or of the current delivered to the individual animals, were provided in the body of the submitted manuscript.

4.2. Outcome of the study under experimental slaughterhouse conditions

4.2.1. Onset of unconsciousness and insensibility

Evaluations of stunning methods require well-controlled studies under laboratory conditions as a first step, to characterise the animals' responses (unconsciousness, absence of pain) using the most sensitive and specific methods available (e.g. electroencephalography (EEG), blood samples) and to establish the correlations between these measurements and non-invasive parameters that can be applied in slaughterhouses (EFSA AHAW Panel, 2013a).

The assessment of the information provided in the submitted study in relation to the onset and duration of unconsciousness and insensibility (Section 3.2.1 of EFSA guidance (EFSA AHAW Panel, 2013a)) is collated in Table 2 of this opinion (Appendix A).

4.2.1.1. Start and end of EEG measurement

The information provided is summarised in Table 2 (Appendix A).

4.2.1.2. EEG measurement

The information provided is summarised in Table 2 (Appendix A).

In the assessment of unconsciousness using EEG frequencies, it is stated that the fast Fourier transformation analysis computed the power content and the predominance of 8 to 13 Hz frequency high-amplitude EEG activity after stunning. However, it is reported that, in order to achieve this frequency, a range of 5 to 15 Hz was used. This is not a measurement practice that is generally accepted for analysing EEGs.

The authors set EEG criteria without citing relevant references or providing validation data.

4.2.1.3. EEG recording and analysis

The information provided is summarised in Table 2 (Appendix A).

4.2.1.4. EEG results

The information provided is summarised in Table 2 (Appendix A). It was noted that there are inadequacies in the reported observations: for example, none of the *Pascual* (13 to 16 kg carcass weight) commercial category lambs stunned head only with 0.3 A showed 5 to 15 Hz epileptiform activity, and only 60 to 67 % of animals subjected to higher current levels (i.e. 0.5, 0.7 and 1.0 A) showed epileptiform activity. The finding that more than 30 % of the animals stunned with 1.0 A failed to show epileptiform EEG activity is too high and does not fulfil the requirements for humane slaughter.

In addition, the time to onset or the duration of tonic–clonic seizures, which are the physical signs of epileptiform activity occurring in the brain, are not reported. Therefore, it is not possible to verify that the epileptiform activity reported in the study is a true representation of the electrical activity originating from the brain and not an artefact.

The time to onset of EEG total power to less than 10 % of its pre-stun value, which is used as an indicator of onset of a quiescent EEG, is rather delayed, i.e. on average 58.9 seconds following HO stunning and slaughter and 51 seconds following HB stunning and slaughter. The reason(s) for the delayed onset is not reported.

The comments outlined in the paragraphs above are also relevant to other categories of lambs; however, those results are inconsistent and seemingly counter-intuitive. For example, more *Recental* (9 to 13 kg carcass weight) commercial category lambs subjected to HO or HB stunning with 1.0 A showed positive corneal reflex and rhythmic breathing than those subjected to lower currents of 0.5 or 0.7 A.

4.2.1.5. Conclusions

The outcomes of the intervention (the EEG results) are inconsistent and contradictory.

4.2.1.6. Animal-based measures to detect onset of unconsciousness

The information is provided (Table 2, Appendix).

Within the *Pascual* category, rhythmic breathing was present in 20 % of HO stunned and 5 % of HB stunned lambs and it persisted until 107 seconds post stunning. In view of the fact that animals were slaughtered by severing both the carotid artery and the jugular vein, one would not expect to see animals showing these reflexes, especially not rhythmic breathing. The presence of rhythmic breathing

indicates that unconsciousness was either not induced by the intervention or of very short duration and that it did not last until death occurred through bleeding (EFSA AHAW Panel, 2013b).

Corneal reflex was present in 50 % of HO stunned and 15 % of HB stunned lambs and it persisted until 88 seconds post stunning. Although the presence of corneal reflex alone does not necessarily indicate the presence of cerebral cortical function associated with consciousness, the presence of corneal reflex and rhythmic breathing simultaneously should be considered as a serious animal welfare concern, according to the previous EFSA opinion on monitoring welfare at slaughter of small ruminants (EFSA AHAW Panel, 2013b). A considerable proportion of animals subjected to HB stunning retained corneal reflex and rhythmic breathing, suggestive of failure to induce unconsciousness and cardiac ventricular fibrillation.

The observations and comments above are also relevant to the other categories of lambs studied. The outcomes of the interventions seem to be different and inconsistent in different lamb categories and kid goats and the reasons for this are not explained in the study.

4.2.2. Absence of pain, distress and suffering until the loss of consciousness and sensibility

Any attempts to stun an animal with a current less than that required for achieving immediate loss of consciousness and sensibility will be painful. The amount of current necessary to induce tremors resembling seizures is less than that required to induce epileptiform activity in the brain, indicative of unconsciousness and insensibility. Therefore, the assessment of the onset of unconsciousness and insensibility by EEG is required to eliminate any uncertainties (EFSA AHAW Panel, 2013a).

Information provided by the submitted study in relation to animal based measures associated with pain, distress and suffering during the induction of unconsciousness (Section 3.2.1 of EFSA AHAW Panel, 2013a) is assessed in Table 3 (Appendix A). No evidence for absence of suffering is presented in the study, in spite of the fact that the interventions were unsuccessful in a considerable proportion of animals, i.e. they failed to induce unconsciousness and/or cardiac ventricular fibrillation.

It was noted that, although EEG was performed, this was not intended to be a measure or an indicator of pain (Table 3, Appendix A).

4.2.3. Duration of unconsciousness and insensibility

HO electrical stunning using the parameters in the submitted study failed to produce immediate loss of consciousness in all the animals, as evidenced by the lack of epileptiform EEG activity, and also did not prevent recovery of consciousness during bleeding in some animals, as evidenced by the presence of corneal reflex and rhythmic breathing. HB electrical stunning also failed to produce immediate onset of generalised epileptiform activity in the EEG indicative of unconsciousness, albeit such an epileptiform activity would be expected to have a shorter duration than that with HO stunning owing to subsequent fibrillation or stopping of the heart.

Electrocardiograms were not recorded in this study to confirm successful induction of cardiac ventricular fibrillation. Nevertheless, the results of behavioural and/or physiological reflexes showed that a considerable proportion of lambs subjected to HB stunning showed signs of consciousness such as rhythmic breathing and corneal reflex (lines 305 to 308 for the *Pascual*, lines 351 to 353 for the *Recental*, and lines 392 to 394 for the *Lechal* (less than 7 kg carcass weight) lamb breed categories).

In contrast, none of the kid goats subjected to HB stunning showed these reflexes. The absence of these reflexes is indicative of successful induction of cardiac ventricular fibrillation in that species. Therefore, HB interventions applied to lambs failed to fulfil requirements in terms of successful induction of cardiac arrest.

In addition, the possibility that restraining and slaughter of animals by neck cutting could have masked the signs of consciousness in animals subjected to inadequate or poor stunning could not be ruled out.

5. Reporting quality

5.1. Assessment of the reporting quality of the submitted study

The assessed studies did not pass the eligibility assessment and, therefore, reporting quality was not assessed (EFSA AHAW Panel, 2013a).

6. Methodological quality

6.1. Quality assessment of the internal validity of the submitted studies

The assessed studies did not pass the eligibility assessment and, therefore, methodological quality was not assessed (EFSA AHAW Panel, 2013a).

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Although the descriptive information provided in the submitted study fulfil the EFSA guidance requirement for reporting the intervention, the animal welfare outcomes raise serious concerns.

The submitted studies were not adequate for a full welfare assessment of the alternative method studied because they do not fulfil the eligibility criteria defined in the EFSA guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions. Reported results indicated inconsistencies of observed outcomes for different animal categories, indicating a need to verify if the stunning equipment delivered the proposed current to individual animals. Such validation measurements were not provided.

The EEG results are inconsistent and contradictory. The HO electrical stunning and slaughter intervention failed to achieve immediate loss of consciousness and/or maintain unconsciousness during bleeding in all of the animals. The HB electrical stunning and slaughter intervention failed to achieve immediate loss of consciousness and cardiac ventricular fibrillation in all the animals, as evidenced by the concurrent presence of corneal reflex and rhythmic breathing. No evidence of absence of pain and suffering is presented in the study.

The stunning parameters proposed in the study (Llonch, Rodríguez, Casal et al.) do not fulfil the animal welfare requirement, which is the successful induction of unconsciousness until the onset of death via slaughter or cardiac ventricular fibrillation.

Following the review of the provided information and evaluation of the stunning procedure applied and its welfare outcome (ToR 1 and ToR 2), it was concluded that the submitted study does not provide enough scientific information upon which to base an assessment of the scientific approach and parameters suggested.

Consequently, a full assessment of the animal welfare implications of the proposed stunning procedure was not undertaken.

RECOMMENDATIONS

When a particular parameter aimed at achieving effective stunning and slaughter without causing avoidable pain and suffering, the procedure should be terminated on ethical and animal welfare grounds.

The use of live animals in experimental procedures should be minimised as far as possible: specifically the 3R principles (replacement, reduction and refinement) shall be considered in accordance with Directive 2010/63/EU.

DOCUMENTATION PROVIDED TO EFSA

1. Llonch P, Rodríguez P, Casal N, Carreras R, Muñoz I, Dalmau A and Velarde A. Electrical stunning effectiveness with current levels lower than 1 A in lambs and kid goats. IRTA, Spain.

2. Rodríguez P, Llonch P, Casal N, Carreras R, Dalmau A and Velarde A. Effect of the electrical stunning with inferior intensities of current to 1 Ampere on the carcass quality in *Pascuales* lambs. IRTA, Spain.

3. Rodríguez P, Llonch P, Casal N, Dalmau A and Velarde A. Effect of electrical stunning with inferior intensities to 1 Ampere on the induction to the unconsciousness in lambs. IRTA, Spain.

REFERENCES

EFSA (European Food Safety Authority), 2004. Welfare aspects of the main systems of stunning and killing the main commercial species of animals. The EFSA Journal 2004, 45, 1–29.

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013a. Guidance on the assessment criteria for studies evaluating the effectiveness of stunning interventions regarding animal protection at the time of killing. EFSA Journal 2013;11(12):3486, 41 pp. doi:10.2903/j.efsa.2013.3486

EFSA AHAW Panel (EFSA Panel on Animal Health and Welfare), 2013b. Scientific Opinion on monitoring procedures at slaughterhouse for sheep and goats. EFSA Journal 2013;11(12):3522, 65 pp. doi:10.2903/j.efsa.2013.3522

APPENDIX

Appendix A. Assessment of eligibility criteria

Table 1: Parameters assessed when applying a stunning intervention based on head-only (HO) and head-to-body (HB) electrical stunning methods, based on Annex I of Council Regulation (EC) No 1099/2009 and on further details of requirements as determined by the EFSA guidance (corresponding to Table 3 (EFSA AHAW Panel, 2013a))

Parameter	Component	Description presented in study (Llonch, Rodríguez, Casal et al.) (all specifications should be in internationally recognised units)	Is the information required by the EFSA guidance present? (yes, no or not applicable)
Minimum current (A or mA)	Current type	Alternating current	Yes
	Waveform	The equipment provided a 50 Hz biphasic sine-wave AC with the capacity to set a constant value for the current modulating the voltage output up to 230 V	Yes
	Minimum current [(a)]	0.3 mA root square means The delivered electric current instantaneously reached the brain with the intensity required for each treatment group (0.3, 0.5, 0.7 and 1.0 A, root square means)	Yes
	Latency	A constant current stunner was used	Not applicable
Minimum voltage (V)	Exposed minimum voltage (V) [(a)]	The minimum root square voltage to which the animal was exposed was recorded at each application	Yes
	Delivered minimum voltage (V) [(a)]		Yes
Frequency (Hz)	Maximum frequency (Hz)	50 Hz	Yes
	Minimum frequency (Hz)	50 Hz	Yes
Minimum time of exposure		3 seconds	Yes
Frequency of calibration of the equipment		All equipment was checked and calibrated daily before starting the experiments according to the factory calibrating recommendations (lines 138–139).	Yes

Parameter	Component	Description presented in study (Llonch, Rodríguez, Casal et al.) (all specifications should be in internationally recognised units)	Is the information required by the EFSA guidance present? (yes, no or not applicable)
Optimisation of the current flow	Electrode characteristics	Either two-electrode scissor-type dry stunning tongs or three-electrode tongs to apply HO or HB stunning, respectively	Yes
	Electrode appearance	Stainless steel electrodes	Yes
	Animal restraining	Sternal recumbency on a V-restrainer straddle/cradle with limbs approximately 3 cm above the ground. During current application, pre-heated (40–50 °C) tap water was sprayed automatically to the three points of contact between the electrodes and the skin.	Yes
Prevention of electrical shocks before stunning		Both systems had a switch to let the current flow in order to prevent electrical shock before stunning (lines 137–138)	Yes
Position and contact surface area of electrodes	Position of the electrodes	In both HO and HB stunning systems, head electrodes were placed between the eyes and the ears on either side of the head. In HB stunning, the third electrode was placed above the spinal cord, behind the position of the heart (lines 143–145)	Yes
	Type of electrodes	The stunning system was connected either to a two-electrode scissor-type dry stunning tongs (PZ004, Gozlin, Modena, Italy) or a three-electrode tongs (2A Handset, Jarvis, Auckland, New Zealand) to apply HO and HB electrical stunning, respectively. The HO system was a grip gun shape made of a plastic material with stainless steel electrodes The HB system consisted of two frontal shaped stainless steel tongs to facilitate penetration of the wool and, at a distance of 35 cm, a third platter stainless steel electrode	Yes
	Animal skin condition	The wool in the frontal region of the head between the ears was shaved/clipped	Yes

(a): Information on mean or median and range and standard deviation or interquartile range to be provided.
(b): In the case of simple stunning.

Table 2: Information provided by the submitted study in relation to the onset and duration of unconsciousness and insensibility (Section 3.2.1 of EFSA AHAW Panel, 2013a)

Parameter	Information provided in the submitted study (Llonch, Rodríguez, Casal et al.)	Is the information required by the EFSA guidance present? (yes, no)
Start and end of EEG measurement	The EEG activity was recorded from 2 minutes before stunning until 5 minutes after the stun application	Yes
EEG measurement	Detailed description in section on EEG of the submitted study	Yes
EEG recording analysis	The EEG files were stored and assessed afterwards by both visual inspection and fast Fourier transformation analysis. A seizure was considered to occur when the post-stun EEG amplitude was from 4 to 8 times greater than that before the stun. The EEG was considered to be quiescent when the power spectrum was less than 10 % of the baseline	Yes
EEG results	The criteria for the EEG parameters were selected after comparing visual inspection and numerical analysis: time of appearance of slow waves, onset of a significant decrease in the EEG power spectrum, suppressed or quiescent EEG	Yes
ABM to detect onset of unconsciousness	Rhythmic breathing, corneal reflex, spontaneous blinking and response to pain and or threatening movements	Yes

ABM: animal-based measure.

Table 3: Information provided by the submitted study in relation to animal-based measures (ABMs) associated with pain, distress and suffering during the induction of unconsciousness (Section 3.2.1 of EFSA AHAW Panel, 2013a)

Response type	Groups of ABMs	Study (Llonch, Rodríguez, Casal et al.) under experimental slaughterhouse conditions	Do the ABMs suggest pain, distress and suffering (yes, no)
Behaviour	Vocalisation	No	No
	Postures and movements	Corneal reflex, spontaneous blinking, response to threatening movements	Yes
	General behaviour	Rhythmic breathing, response to pain	Yes
Physiological response	Hormone concentration	No	No
	Blood metabolites	No	No
	Autonomic responses	No	No
Neurological response	Brain activity	EEG	No

ABBREVIATIONS

A	Amperes
ABM	animal-based measure
AHAW Panel	EFSA Panel on Animal Health and Welfare
EC	European Commission
EFSA	European Food Safety Authority
EEG	electroencephalogram
HB	head-to-body electrical stunning
HO	head-only electrical stunning
ToR	terms of reference provided by the European Commission