A method to assess unconsciousness and insensitivity in eel (*Anguilla Anguilla*, L.) for development of humane slaughter methods

J.W. van de Vis[1], E. Lambooij[2], C. Pieterse[2] and R.J. Kloosterboer[1]*

* To whom correspondence should be addressed.

[1]Netherlands Institute for Fisheries Research (RIVO), P.O. Box 68, 1970 AB IJmuiden, The Netherlands
[2]Institute for Animal Science and Health (ID-Lelystad), P.O. Box 65, 8200 AB Lelystad, The Netherlands

Summary

In Europe consumers have become aware of quality, including a humane production of animals. The awareness led farmers and processors to pay more attention to humaneness of methods for slaughter of fish. In the Netherlands retailers are beginning to make demands upon slaughter of eel. The term slaughter is used in practice to designate the first step in the process, which is applied to convert a live animal into food.

Application of a humane slaughter method should result in immediate unconsciousness and insensitivity, which last until death. When it is not possible to induce immediate unconsciousness and insensitivity the fish should be spared avoidable stress, pain and discomfort. The animal is rendered unconscious and insensitive by stunning.

The objective of the study was to develop a method for evaluation of consciousness and sensitivity of eel (*Anguilla anguilla*, L.). A complete evaluation of slaughter methods also requires assessment of stress, which may occur in fish prior to stunning. Assessment of stress was not part of this study.

A method to record SERs in the EEG has been developed for eel. It was established that an eel of 700-800 g can be rendered unconscious and insensitive immediately by passing a 545 mA current, 50 Hz AC, through the brains for 1.2 second. The period of unconsciousness and insensitivity lasted for at least 30 seconds. Electrical stunning caused a temporary cardiac dysfunction in eel, as shown on the ECG.

The period of unconsciousness and insensitivity can be prolonged for an eel by using a combination of a peak current stun (3 s on average 250 V, 50 Hz AC) and long duration low current stun (on average 50 V, 50 Hz AC for 300 s), applied head-tail. It is possible that the eels will not recover, as the heart activity and activity of the muscles used for breathing are both stopped, resulting in anoxia in the brains. It is foreseen that this approach can be used in practice to render eels unconscious and insensitive immediately and permanently at slaughter.

Introduction

In slaughter animals (including fish) the welfare *ante mortem* and the quality of the meat or flesh *post mortem* are easily adversely affected by farming conditions and harvest conditions. Farming conditions comprise amongst others water quality and stocking density. Harvest of fish consists of crowding and catching, sorting, transport, lairage (i.e. keeping the animals in e.g. a tank before they are slaughtered) and slaughter. It is known that harvest is one of the most intense stressors in fish farming (Thomas et al., 1999). Harvest is the process in aquaculture whereby a live ani-

mal is converted into an edible product. It is the key time when both product quality and welfare can be affected. Fish welfare is a relatively new concept. However, in case of mammals the concept of animal welfare has gained acceptance. On basis of similarities in basic structure of neurones and neuronal biochemistry to that of mammals and similarities in stress responses and behaviour to higher vertebrates it is likely that welfare of fish can be affected, especially at harvest (Clark and Squire, 1988; Kestin et al., 1991, 1995; Kestin, 1994; Overmier and Hollis, 1990; Verheijen and Flight, 1997; Wiepkema, 1997).

In the case of fish there is no EU legislation for the protection of the animals at the time of slaughter (Council Directive 93/119 EC, 1993). For red and white meat animals it is generally stated that unconsciousness and insensitivity should be induced as soon as possible without a detrimental effect on the welfare of the animal and the meat quality of the carcass (Blackmore & Delany, 1988). This can be used as basis to propose general terms of reference for the evaluation and development of slaughter methods for fish. The general terms of reference are met when stunning induces instantaneous unconsciousness and insensitivity in fish or, when this is not possible, the animal should be rendered unconscious and insensitive without avoidable stress, pain or discomfort.

The current slaughter process in the Netherlands consists of placing live eels in a so-called salt bath for desliming. In the next steps the eels are washed with tap water and gutted. Subsequently, the eels are processed. A few electro-physiological and behavioural studies have been performed to determine the onset of unconsciousness and insensitivity at slaughter. It was observed that the eels might not be rendered unconscious and insensitive instantaneously. Moreover, the eels showed vigorous movements as response to the salt bath (Verheijen and Flight, 1997; Van de Vis et al., 2000).

Current methods to indicate consciousness and sensitivity in slaughter animals consist of recording the EEG (electroencephalogram) and somatosensory evoked responses (SERs) or visual evoked responses (VERs) in the EEG.

In recent studies VERs in the EEG were recorded to assess consciousness and sensitivity in fish (Kestin et al., 1991; Van de Vis et al., 2000). However, it is possible that effective electrical stunning does not result in loss of VERs. In a publication of Gregory and Wotton (1984) it was concluded that bursts of visual evoked cortical responsiveness were observed during the epileptiform phase, which was induced in sheep. When the epileptiform phase is registered on the EEG an animal is considered to be unconscious and insensitive.

In red and white meat animals the induction of unconsciousness and insensitivity is assessed by recording the EEG and SERs in the EEG. Changes in the pattern of the EEG may indicate unconsciousness. A SER is the response to a pain stimulus. Registration of the SERs is of interest as the application of a killing method may give pain stimuli to the animal.

The ECG (electrocardiogram) is record-

ed to assess cardiac dysfunction (i.e. fibrillation or arrest) at stunning. Gitter (1933) recorded an ECG for the first time in eel.

Cardiac arrest results in cessation of the supply of oxygenated blood to the brain. The cessation may eliminate the potential problem of resumption of consciousness in an animal at application of a killing method.

The objective of the proposed study was to develop a method for evaluation of consciousness and sensitivity in eel (*Anguilla anguilla*, L.). A complete evaluation of slaughter methods also requires assessment of stress, which may occur in fish prior to stunning. Assessment of stress was not part of this study.

Materials and Methods

Eels with a live weight of 700 to 800 g were used. Seven days before the experiment the required number of eels were fasted and delivered at the laboratory. At the laboratory the eels were placed in a tank with tap water of 13°C.

On the day of the experiment the eels were placed one by one in a special developed restrainer, which was placed on a table. This restrainer consists of two halves of a tube of 20 cm, which were fixed together by rubber bands. The lower jaw of the eel was fixed by a hook and the tail was placed in a wide Plexiglass tube. Prior to stunning, the eel was equipped with EEG and ECG electrodes. The 10 mm long silver spiked EEG electrodes were positioned in the head using a modified air nailer. The positions of the electrodes in the brains are described by Kestin et al., 1991. The steel ECG electrodes were placed under the skin at the base of the spinal column close to the implantation of the pectoral fins. The earth electrode for both the EEG and ECG was the hook placed in the lower jaw. The EEG and ECG were recorded during 1 minute before the application of electricity. Immediately after stunning the EEG and ECG were recorded for 2 minutes. An Elema Schönander (Sweden) recorder was used for registration of the EEG and ECG. The responses to pain stimuli by scratching the skin of the tail with a needle were checked on the EEG (i.e. SERs). Simultaneous with the registration of the EEG and ECG the behaviour was recorded on video.

During the application of electricity the EEG and ECG recordings were blocked and the conditions used (voltage, amperage and time in seconds) were recorded.

Two electrical stunning methods were applied on individual eels. The eels from the first method, which were given a recovery period of 10 min after the application of the head-only stun, were also used for the whole body stun.

Head-only stun. In the first method current was delivered via tongs with spiked electrodes (scissors-model) during approximately 1 s. The electrodes were placed on the head between the eye and the opening of the gill. A stunning apparatus, which was set at a constant voltage of 250 V (50 Hz AC), was used.

Whole body stun. In the second method the eel was stunned by 250 V (50 Hz AC), applied during approximately 3 s, which was followed immediately by a 50 V (50 Hz AC) stun during 5 minutes with spiked

electrodes on the head and tail. Another type of electrode was placed on the tail, compared with the electrode on the head.

Results and discussion

Aquaculture is growing in importance throughout the European Community. At present most research has been directed at increasing the production, with little emphasis on welfare. However, as the industry matures, the quality, including welfare, becomes increasingly important. In addition, concern for animal welfare is increasing, especially at harvest. Furthermore, in bird and mammal slaughterhouses it has been observed that in many cases improvements in animal welfare can lead to improvements in meat quality. There are preliminary indications that this may also apply to fish. It is therefore timely that the improved methods for assessment of slaughter methods become available for development of stunning methods to reduce stress in fish at killing.

Head-only stun. In the first method individual eels were stunned head-only with 255 V on average. It was observed that stunning at lower voltages may not always render every eel unconscious and insensitive immediately (results not shown). The average amperage and duration were 545 mA and 1.2 s, respectively. The duration of the tonic/clonic phase on the EEG was on average 11 s. After stunning the ECG revealed a temporary fibrillation.

The tonic/clonic phase is part of a general epileptiform insult on the EEG. The general epileptiform insult on the EEG was characterised by a tonic/clonic phase and an exhaustion phase. Such an insult on the EEG was characterised in sheep in the following ways: the relatively small waves (initial phase) grew irregularly (tonic phase), with an irregular increase in amplitude and decrease in frequency (clonic phase) followed by a phase of strong depression of electrical activity (exhaustion phase) (Lambooij, 1981). During these phases an animal is considered to be unconscious and insensitive (Lambooij, 1981).

The shortest general epileptiform insult was 30 s on the EEG. This was also observed in behaviour, as the eel reacted in behaviour and on the EEG to needle scratches (i.e. a SER) at 30 s. The other eels were only able to respond on the EEG and with respect to behaviour after substantially more than 30 s (results not shown).

Whole body stun. The second method consisted of whole body stunning of the individual eel by application of a voltage and amperage of on average 253 V and 128 mA for 3 s followed by 53 V and 13 mA during 300 s. The current, which was applied at peak stunning was lower than expected. This was probably due to the use of another type of electrode, which was placed on the tail, compared with the electrode on the head.

After the peak stun in combination with a low current long duration stun the EEG tended to an iso-electric line (i.e. the brain activity is absent) on the EEG and no responses on the EEG and in behaviour to needle scratches were observed after stunning. It is possible that the eels will not recover as the heart activity and activity of

the muscles used for breathing are both stopped, resulting in anoxia in the brains.

However, it is possible that the current of 128 mA, which was passed through the brains for 3 seconds, was too low to induce immediate unconsciousness and insensitivity. Therefore, it is recommended that a current of at least 545 mA is applied for the peak stun.

More details on the general epileptiform insult in eels with respect to the EEG and behaviour, and ECG after the application of electrical stunning methods will be given in a future publication.

Conclusions

In conclusion it can be stated that:

- Registration of the EEG, SERs and ECG are methods for evaluation of slaughter methods applied on eel.
- An individual eel might be effectively stunned with an average current of 545 mA (at 255 V on average) for 1.2 second. However, most eels recover and, therefore, the method as such cannot be used for humane slaughter of eels in practice.
- The period of unconsciousness and insensitivity can be prolonged by using a combination of peak current stun and a long duration low current stun, applied head-tail on an eel. It is possible that the eels will not recover as the heart activity and activity of the muscles used for breathing are both stopped, resulting in anoxia in the brains. It is foreseen that this approach can be used in practice to render eels unconscious and insensitive immediately and permanently during slaughter.

Acknowledgements

The study was financed by the EU, contract FAIR CT97-3127, and the Dutch Ministry of Agriculture, Nature Management and Fisheries.

The authors want to thank Mr. J.W.H. van der Heul from RIVO for managing the facilities at the laboratory.

References

Blackmore, D.K. and Delany, M.W., 1988. Slaughter of stock. *Publ. No. 118. Vet. Con. Ed. Massey University*. Palmerston North, New Zealand: Vet. Con. Ed. Massey University.

Clark, R.E. and Squire, L.R., 1998. Classical conditioning and brain systems: The role of awareness. *Science*, 280, 77-81.

Council Directive on the protection of animals at the time of slaughter or killing 1993. *Official J. Eur. Comm.* No 1.340/21.

Gitter, A., 1933. Untersuchungen über die Herztätigkeit der Fische. *V.Z. vergl. Physiol.*, 18, 654-666.

Gregory, N.G. and Wotton, S.B., 1984. *Sheep slaughtering procedures 4. Responsiveness of the brain following electrical stunning.* Report of the Agricultural Research Council, Meat Research Institute, Langford, Bristol BS18 7DY.

Kestin, S.C., 1994. *Pain and stress in fish.* Royal Society for the Prevention of Cruelty to Animals.

Kestin, S.C., Wotton, S.B. and Gregory, N.G., 1991. Effect of slaughter by removal from water on visual evoked activity in the brain and reflex movement of rainbow trout. *Vet. Rec.* 128, 443-446.

Kestin, S.C., Wotton, S.B. and Adams, S., 1995. The effect of CO_2, concussion or electrical stunning of rainbow trout on fish welfare. *In*: Scheire, K., Apselagh, L. and H. Jonkeere, H., (eds.). *Quality in aquaculture* Int. Conf. Aquaculture Eur. '95, Trondheim, Norway. pp 380-381.

Lambooij, E., 1981. *Some neural and physiological aspects of electrical and mechanical stunning in ruminants*. Ph.D. thesis, State University of Utrecht.

Overmier, J.B. and Hollis, K.L., 1990. Fish in the think tank: Learning, memory and integrated behaviour. *In*: Kesner, R.P., and Olson, D.S. (eds.). Neurobiology of Comparative Cognition, Hillsdale (NJ): Lawrence Erlbaum, pp 205-236.

Thomas, P.M., Pankhurst, N.W. and Bremner, H.A., 1999. The effect of stress and exercise on post-mortem biochemistry of Atlantic salmon and rainbow trout. *J. Fish Biol.*, 54, 1177-1196.

Van de Vis, J. W., Oehlenschläger, J., Kuhlmann, H.,

Münkner, W., Robb, D.F.H. and Schelvis-Smit, A.A.M., 2000. Commercial and experimental slaughter of eel (*Anguilla anguilla*, L.): effect on quality and welfare. *In*: Kestin, S.C. and Warris, P. (eds.). *The Proceedings of the International Farmed Fish Quality Conference*, Bristol, UK, April 1999, in press.

Verheijen, F.J. and Flight, W.F.G., 1997. Decapitation and brining: experimental tests show that after these commercial methods for slaughtering eel *Anguilla anguilla* (L.), death is not instantaneous. *Aquaculture Res.*, 28, 361-366.

Wiepkema, P.R., 1997. The emotional vertebrate. *In*: Dol, M., Kasanmoentalib, S., Lijmbach, S., Rivas E. and Van den Bos, R. (eds.). *Animal Consciousness and Animal Ethics*. Van Gorcum & Comp B.V., Assen, The Netherlands, pp. 93-102.