

## Poster Abstracts

### **Risk assessment for beef cattle at slaughter: a method for performing a risk assessment based on empirical**

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**Background:** Risk assessment (RA) for animal welfare is a systematic process that uses modelling to estimate the likelihood of poor welfare effects occurring from exposure to hazards. Existing RA for animal welfare mostly rely on qualitative data and/or expert opinion to perform hazard identification, exposure assessment (ie assessment of hazard likelihood to occur) and adverse effect characterisation (ie estimate of severity, duration and likelihood of the adverse effects due to hazards). **Objectives:** To develop a method for performing a RA for beef cattle at slaughter based on empirical data. **Methods:** 56 hazards potentially relevant to beef cattle at slaughter and indicators of poor welfare were identified via a literature review. Observations of 1,171 beef cattle in three slaughter plants in northern Italy were performed, to assess the relevance of the hazards and indicators of poor welfare identified, define them precisely and standardise their measurement, and develop RA-tailored data collection forms. The indicators of poor welfare were associated to different severity classes defined qualitatively on the basis of the intensity of the behavioural responses of the animal, using a 1–4 scale (ie limited severity, moderate, severe and critical). This allowed the simultaneous identification of the adverse effects and their severity, every time an indicator of poor welfare was detected. A second series of observations was performed on a sample of 1,427 animals in one abattoir. On-site exposure assessment and adverse effect characterisation were estimated per phase of the slaughter process and, for pre-slaughter handling phases (ie unloading; move along the corridors; and at the entrance of the stunning box), they were performed in two steps (ie first at group level and subsequently individually). At stunning/killing the animals were observed individually. **Results:** Exposure assessment corresponded to the observed occurrence of the hazards; adverse effect characterisation was estimated by detecting the indicators of poor welfare and thus assessing the occurrence of the adverse effects by severity levels. Confidence intervals of the estimates were calculated to reflect the uncertainty. The results of the group observations were corrected using the data collected at individual level and discarded when considered not reliable. **Conclusions:** a novel method for performing RA for cattle welfare at slaughter based on empirical data was developed, which may show useful implications for risk management purposes in slaughter plants and for a comparison to RA based on expert opinion, to assess to which extent the latter RA reflects the field conditions.

### **Official control of animal welfare at slaughter**

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Quite substantial research has been carried out in relation to animal welfare at slaughterhouses, both concerning lairage and handling facility design and concerning the operation of different stunning techniques. However, very little has been written about the official control of animal welfare at commercial slaughterhouses. During 2009, the Swedish National Food Administration (CCA for official control at slaughterhouses) together with the Swedish Board of Agriculture (CCA for animal welfare) and the Swedish University of Agricultural Sciences initiated a nationwide survey of animal welfare at commercial slaughterhouses. A total of 23 major slaughterhouses, of which 12 slaughtered beef cattle, 10 sheep, 11 pigs and 7 poultry, were included in the study. The aim of the study was to make a baseline survey of the animal welfare situation at the slaughterhouses, and to further develop and standardise the official controls. This included the evaluation of a number of given animal welfare indicators and measurement criteria. The official veterinarians were instructed to score a number of animal welfare parameters for a set number of animals or during a set amount of time per day. Overall, these animal welfare parameters were evaluated by the official vets at 10 different instances (15 for poultry) at each of the slaughterhouses in the study, over a period of six months. Examples of included parameters are: animals falling or vocalising at unloading, use of rough coercion methods, stocking density during lairage, proportion of animals kept in lairage over night, stun quality, stun-stick interval, skin lesions, and fractures or bruises on the carcasses. Overall, compliance with national and EU-related animal welfare legislation was good. The results indicate that approximately 40% of the cattle and pigs and 60% of the sheep were delivered to the slaughterhouse the day before slaughter. Broilers spent on average 8 h in containers, from catching to unloading. Stocking density regulations for lairage were, with few exceptions, generally complied with. The percentage of dead-on-arrival broilers was low and varied from 0.0 to 0.7%, indicating substantial differences between transporters/slaughterhouses. Relatively high percentages, approximately 30%, of rough coercion methods (by definition including any use of electric goads), were reported from several cattle slaughterhouses. Rough coercion was not common at pig slaughter plants, except at one site. The stun quality was generally found to be adequate, regardless of species and method applied. The maximum stun-stick interval for sheep (20 s) was, however, exceeded for 18% of the animals.

### **How to improve the welfare of farmed fish at slaughter: the impact of legislation**

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Aquaculture is Norway's major food production industry. In 2009, Norway slaughtered an estimated 172 million salmon (*Salmo salar*) and 25 million rainbow trout (*Oncorhynchus mykiss*). As fish are covered by the Animal Welfare Act, their welfare must be considered during all stages of production. A legislative approach allows mandatory requirements to be used to safeguard all farmed fish. These can be enforced in cases of non-compliance. Work on new fish welfare legislation started in January 2002 and the Regulations concerning abattoir and processing plants for aquaculture came into force on 1 January 2007. The involvement of industry, animal welfare organisations, academia and other NGOs is essential for creating an understanding for why animal welfare is important. A solid scientific basis increases the credibility of legislation and is equally valuable. Such a process necessarily takes time, but it ensures that relevant animal welfare issues are addressed and that the mandatory requirements are practicable. A ban on the use of carbon dioxide for stunning purposes was proposed, based on scientific evidence that it does not result in immediate loss of consciousness and causes considerable stress. As almost 90% of the abattoirs at that time (2005/2006) still used carbon dioxide it was decided that the ban on the use of CO<sub>2</sub> should be delayed until 1 July 2008. The length of the transitional period was discussed with the industry to make certain that it would be possible for them to adjust to the new requirements within the given time-frame. Even so, the ban has had to be postponed twice. Despite this, only about 20% of the abattoirs still used carbon dioxide at the end of 2010. The others have installed either percussive or electrical-stunning equipment. Research into fish welfare has increased since the authorities started working on the legislation, and especially since the ban on CO<sub>2</sub> was first mentioned. There have been major advances in the development of suitable alternative technology in recent years. This shift to more humane stunning methods would not have progressed so far or have been possible without the legislative requirements that are now in place.

### **Assessment of road transport of farmed African catfish and European eel with respect to animal welfare**

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Aggression is a problem in African catfish (*Clarias gariepinus*) and eel (*Anguilla anguilla*) aquaculture; it is provoked by commercial practices. Welfare of these species can be improved when knowledge on sorting, (re-)feeding and transportation strategies is obtained. The objective was to assess effects of transport of market-size catfish (1.5 kg) and eel (0.13 kg) in The Netherlands with respect to welfare. To assess effects of transport, fish were returned to the premises after transportation. Here, transported fish and non-transported fish (controls) were kept in flow-through systems and monitored for 3 days. Commercial conditions were applied. Catfish: Catfish were placed in flow-through tanks containing spring water (20–23°C, pH 7.3) and fasted for 3 days, at the fish farm. At the day of transport, tanks were drained and the fish were dropped into empty containers and subsequently dropped into water-filled transportation containers, which were placed in an insulated truck. Fish density increased from 250 kg m<sup>-3</sup> (husbandry and fasting) to 500 kg m<sup>-3</sup> (transportation). During transportation, fish were not provided with temperature control, water circulation for purification, oxygenation of their water and the containers were not secured within the cargo area. The duration of transport was 3 h to take the fish from and back to the farm. Eel: Before transport, commercially farmed eels were acclimatised and fed for 3 weeks at our laboratory (25°C, pH 5.5). Before transport, the fish were fasted for four days during which they were kept in flow-through tanks with Nijmegen tapwater (18–19°C, pH 8). At the day of transport they were netted and placed in large buckets and drained. The animals were dropped in transport tanks filled with water. During the entire process the fish density increased from 150 kg m<sup>-3</sup> (husbandry) to 450 kg m<sup>-3</sup> (fasting) and reduced to 300 kg m<sup>-3</sup> during transport. During transportation pure oxygen was added to the water in insulated tanks mounted on a trailer; there was no water circulation for purification. The duration of the transport was 3 h after which the eels were returned to our laboratory.

The results show that transport of African catfish under these commercial conditions causes lasting stress. This is reflected in changes in plasma parameters (cortisol, glucose, NEFA), differences in gill morphology and an increase in recovery time compared to control fish. The eel experiment is under analysis at the time of abstract submission.

### **Relationships between slaughter procedures and reactions of cattle: a field study**

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This study was carried out in a commercial abattoir to evaluate the slaughter procedures used and the cattle's reactions to these procedures. Behavioural observations were carried out from the moment of arrival of animals until bleeding. Several results indicate that all animals were not subjected to the same procedures. As males were perceived as more reactive, they were slaughtered first and consequently, due to time constraints, halal slaughter of some females could be postponed until the next day, or even later, leading to increased lairage times for females (47.6 [± 7.8] h). For the same reason, to limit physical interactions, more males were simultaneously introduced in the slaughter corridor. Consequently, males spent more time in the corridor (12.7 [± 1.2] min) than females (8.8 [± 1.6] min). Overall, these results show that organisational and practical constraints influence the way animals are treated, with possible negative consequences for animal welfare. Overall, animals received between 0 and 26 electrical prods. This large variation was related to the repeated use of the prod on single animals. When calculated per unit of time, introduction of the animal in the stunning box required most prods (15.9 [± 1.5] prods per animal per min) due to balking, probably caused by the vision of humans and a change in flooring. These results suggest that introducing animals in the stunning box induced more fear than other slaughter procedures. Results show further that reactions to aspects of the slaughter procedures could have consequences for subsequent stages. Thus, animals that were more difficult to introduce into the stunning box were more likely to receive two stunning shots. Finally, following stunning or halal slaughter, despite the abolition of the corneal reflex, some animals showed head-raising movements. In addition to its scientific aims, the present study aimed to identify which simple practical modifications could improve animal welfare: homogenisation of the floor at the entrance of the stunning box, increasing lighting in the halal corridor, installation of flexible shields to prevent cattle from seeing people while not obstructing human passages, installation of anti-back-up devices, and training of personnel to limit the use of the prod. Overall, this study presents concrete data on the functioning of an abattoir and cattle reactions to slaughter procedures with a direct interest for the abattoir itself but also for scientific purposes.

### **Humane slaughter of crustaceans**

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The civilised world recognises that many animals, like human beings, are able to feel pain and distress. As a consequence, legislation requires food animals to be killed humanely. This usually means that they are stunned insensible, often by an electrical stun, before being killed so as to ensure that the process of killing involves the least pain and distress. If one were to accept that decapod crustaceans have the ability to experience aversive sensation and feeling known as pain, as recent research appears to indicate (Elwood *et al* 2007, 2009) the question arises as to what is the best and quickest method of rendering these animals insensible prior to killing and whether, given its diffuse nervous system, such crustaceans can be electro-stunned into insensibility or even killed. Research (Roth & Oines 2010)<sup>1</sup> having examined all current methods, recommended electro-stunning as the quickest and most humane. Initial research conducted at Bristol University (Robb 1999)<sup>2</sup> established the methodology and currents required to achieve anaesthesia in a variety of decapod crustaceans by the delivery of a current of 1 A in the case of lobsters and langoustines and 1.29 A in the case of crabs at 50 Hz–110 V. delivered through the medium of a wet sponge to the dorsal shell of the animal whilst semi-submerged in brine. This research resulted in the construction of equipment — Crustastun — a steel box structure with a sprung steel base immersed in brine onto which the crustacean is placed, which when the lid is closed, delivers by the press of a button (controlled by a timer) the required current through a steel plate electrode embedded in the lid and covered with a sponge. Research (Sparrey 2005)<sup>3</sup> demonstrated that the required stun currents were delivered to the crustaceans in less than 0.5 s and that the prolonged application of the current (5 s for lobsters and the smaller decapods and 10 s for the larger edible crabs) resulted in their ultimate death. Research (Neil 2008, 2010)<sup>4</sup> demonstrated that the stun immediately silenced the CNS and PNS of the animals so that no sensory stimuli could pass up or down from the 'brain' of the animal and that the process advanced the breakdown of ATP to IMP which gives the enhanced taste to the meat.

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### **Welfare at slaughter plants processing beef for the internal market in the Argentinean pampas**

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The welfare of livestock animals depends on the attitudes and handling procedures used by farmers, hauliers and abattoir personnel, as well as on transport logistics. Studies of meat pH and bruises detected on carcasses at the slaughterhouse, provide useful information about the traumatic situations the animals endure during the pre-slaughter period. The potential for developing countries to develop trade in markets with higher welfare standards has been highlighted by the OIE. However, less attention has been paid to the welfare of animals produced for internal markets. As a result, the welfare of the animals used for export may tend to be safeguarded more than animals consumed locally. This study aimed to assess the prevalence of carcass bruising and pH<sub>ult</sub> at five abattoirs located in the Argentinean Pampas (Córdoba) that slaughter cattle for the internal market, to quantify possible relations with animal characteristics, origin and selling method. In each slaughter plant, we assessed transport, animal origin, unloading and lairage conditions (1st day), stunning conditions, bleeding and carcass bruising and meat pH (2nd day). Descriptive statistics were calculated and the effect of animal origin (auction market or farm), sex and other characteristics were assessed. A total of 1,084 carcasses were evaluated and 74% presented some type of injury or bruises. All lesions observed were acute, indicating welfare problems in short-term pre-slaughter handling. The animals from cattle fair had significantly more injuries than those coming directly from the farm (67 vs 33%). There was no effect on meat pH which was within the normal range for commercial meats (5.54 [± 0.14]). The average number of shots per stunning was 1.85 (± 0.38). There was a significant effect of animal origin (auction markets: 2.01 [± 0.11]; farm: 1.48 [± 0.40]). The average time between stunning and bleeding was 51' with no differences between classes. Injuries were mainly on the back and on hindquarter (24 and 23.3%, respectively), anatomical parts with high economic value. The results indicate that there are welfare problems during pre-slaughter handling, especially with animals from auction markets. To preserve welfare during handling and slaughter, personnel must pay attention to proper procedure and supervise and train employees.

### **Sensibility in non-stunned calves during slaughter**

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The duration of sensibility during slaughter of calves, which had not been stunned, was the principle clinical observation recorded during the first author's work as an Official Veterinarian in an EU-approved meat plant. A hundred Holstein calves between three and eight weeks of age were observed over a period of four months in cohorts of seven to ten animals. The duration of sensibility was measured with a stopwatch, where practicable without interfering with clinical or animal welfare responsibilities within the constraints of the legislation. The clinical assessment of the signs of consciousness, involved testing the palpebral reflex in particular, in addition the corneal reflex, eyeball movements were observed, pupillary dilatation, breathing, general muscular movements, muscular tone, particularly the tone of the neck muscles, checking if the head had become floppy, righting reflex, physical collapse, strength of heart beat, heart rate, the perfusion of membranes and occlusion of the cardiac extremities of the carotid arteries, were all clinically assessed during the slaughter process to establish physiological status and sensibility of each calf. The loss of sensibility is the legal requirement which must be met, before any calf can be swung aloft by a hind limb. Calves took over 100 s on average to reach insensibility which was judged by the loss of the following reflexes and signs; the palpebral reflex, the corneal reflex, physical collapse, the floppiness of the head, the absence of a righting reflex, the occurrence of an agonal gasp and pupillary dilation. The loss of sensibility in some took three minutes, one calf took five minutes and another took over six minutes, which consisted of initially wriggling free of the manual restraint on the cradle, standing upright for 180 s or longer then lying down in normal bovine recumbency but displaying a strong righting reflex before eventually stretching out into lateral recumbency and losing sensibility as judged by the above criteria. It appeared when some calves were hoisted by a hind leg that sensibility was re-established. It also was observed, when the calves struggled during manual restraint on the cradle, that this may have hastened insensibility. The least natural position of the animal's head during manual restraint appeared to reduce the period of sensibility and hasten death. It appeared that 'active' forms of manual restraint against the calf's strug-



gling, as distinct from 'passive' forms of gentle restraint were accelerators of death. The plant staff endeavoured to ameliorate the animals' plights where possible. The lack of adipose tissue, in the neck area of these calves, allowed a clear view of the cardiac extremities of the severed carotids which were ballooned/occluding from forty seconds post cut. It must be pointed out that the ritual slaughtering in all these groups was of the highest skill with the sharpest knives. The responses of the carotid arteries was presumed to be due to the intrinsic elastic properties of the vessels and not due to operator failure. The anatomy and physiology, particularly of the vascular supply to the brain, may be the explanation of the long duration of sensibility in calves after severing the carotids and other vessels. This leaves the untouched vertebral artery supplying arterial blood to the basioccipital plexus via the carotid rete mirabile and on to the Circle of Willis through the internal carotid. The observation of prolonged sensibility in calves seen here, was predicted by others on the basis of the study of comparative anatomy, physiology and CNS responses to ventral neck cuts. The latter, combined with the findings of prolonged sensibility outlined here, raise questions about the humanness of this type of slaughter.

#### **Transport duration in lambs: effect on animal welfare and meat quality**

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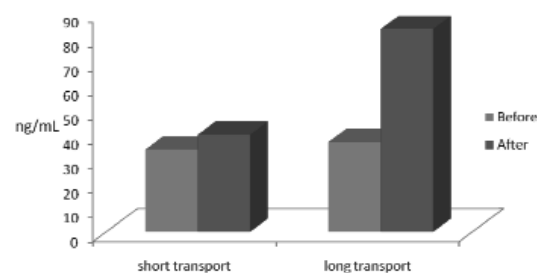
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Transport before slaughter has been stated to compromise animal welfare and consequently meat quality in lambs. To determine the effect of the transport duration on the meat quality 40 lambs (Comisana breed) of 14–16 weeks old were taken and divided into two groups of 20 each. One group was submitted to a short (1 h) transport (ST) and the other one to a long (24 h) transport (LT) both in the same truck and arriving at the same time to the same slaughterhouse. The effect on blood parameters (AST, PT, LDH, BUN, CK, CREA), on salivary cortisol, metabolites of cortisol in faeces and meat quality (pH, conductivity, expressed juice, colour and shear force) were assessed. The duration of transport had no significant effect ( $P > 0.05$ ) on the concentration of cortisol in saliva. In contrast, when samples of faecal cortisol were analysed there was a transport effect ( $P > 0.05$ ). The lambs transported for 24 h showed higher values of cortisol metabolites than those transported for an hour (83 vs 40 ng ml<sup>-1</sup>; Figure 1). This confirms that there is a cumulative effect of stress (chronic stress) when the lambs are subjected to long transport.

However, except in the case of LDH ( $P < 0.01$ ), the others parameters were not different between treatments. In LT, lambs showed lower LDH values than the lambs of ST, although it should be noted that they were within the sheep reference values. The duration of transport did not affect pH, lightness (L\*), the tendency to yellow (b\*) and the ability to retain water of meat. However, there was an effect on the colour attribute of a\* (red trend) and tenderness or maximum shear force. The meat of lambs in the LT was harder and red compared with that of lambs in the ST.

**Figure 1** Faecal cortisol metabolites before and after the long and short transport.



#### **Evaluation of hypobaric hypoxia euthanasia for healthy and moribund nursery pigs**

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Fifty-eight weaned nursery pigs (5.6 [± 1.3] kg) were utilised to compare physiological parameters of hypobaric hypoxia (HH) and carbon dioxide (CO<sub>2</sub>) euthanasia. This experiment was conducted as a completely randomised design using a 2 × 2 factorial arrangement of treatments using a 0.91 m<sup>3</sup> chamber. Factors included euthanasia method: A) hypobaric hypoxia (approximate ascension of 36.9 m s<sup>-1</sup>) or B) CO<sub>2</sub> gas (induction of approximately 20%

of the chamber volume per minute) and 2) health status: A) healthy or B) moribund. Health classification was determined by a certified veterinarian. Two pigs at a time were euthanised for each method by health status treatment ( $n = 8$ , CO<sub>2</sub>-moribund;  $n = 8$ , CO<sub>2</sub>-healthy;  $n = 8$ , HH-moribund;  $n = 5$ , HH-healthy). Jugular blood samples were obtained from each pig 24 h prior to euthanasia. Animals were fitted with ECG- and EEG-monitoring devices, placed in the chamber and kept in the chamber until death was confirmed via ECG and EEG. In addition to ECG and EEG measurements, behavioural parameters were measured and necropsies were performed. The average treatment times were HH, 27.4 ( $\pm 6.7$ ) min and CO<sub>2</sub>, 13.8 ( $\pm 5.1$ ) min. Post euthanasia, a blood sample was obtained from each pig. Blood samples were analysed for lactate ([LAC]), glucose, ionised calcium (iCa), potassium, haemoglobin, and sodium concentrations, haematocrit, pH and partial pressures of oxygen ( $pO_2$ ) and carbon dioxide ( $pCO_2$ ). A health status effect was observed for haematocrit, haemoglobin, iCa, [LAC] and sodium ( $P \leq 0.004$ ). Moribund pigs had higher haemoglobin and sodium concentrations, and haematocrit than healthy pigs. Results indicated a significant interaction between sample time (pre-euthanasia vs post-euthanasia) and treatment (HH vs CO<sub>2</sub>) for [LAC], glucose, haematocrit, haemoglobin, iCa, potassium, sodium and  $pCO_2$  ( $P \leq 0.03$ ). Post-euthanasia glucose concentrations were higher ( $P < 0.0001$ ) in pigs euthanised via CO<sub>2</sub> compared to HH-euthanised pigs. However, post-euthanasia haematocrit, haemoglobin concentration, [LAC] and pH were higher ( $P = 0.03$ ) in HH compared to CO<sub>2</sub>-euthanised pigs. Higher concentrations of haemoglobin and haematocrit are associated with exposure to high altitudes. Higher glucose and [LAC] concentrations may be attributed to differences in length of time the animals remained in the chamber thus altering concentrations of post mortem metabolites. Further research should be conducted to investigate the impact of euthanasia method on physiological, neurological and behavioral parameters.

### **Survey on the actual animal welfare situation at Turkish slaughterhouses**

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Turkey is one of the candidate countries for full EU membership. Therefore, most of Turkish animal welfare legislation is going to be adapted to EU rules including the 'Regulation on the protection of animals at the time of slaughter and killing'. Presently, little is known how

effective this regulation is applied in practice in slaughterhouses in Turkey. This paper reports about the first scientific investigation on the actual animal welfare situation in Turkish slaughterhouses and compares the findings and observations in practice with the animal welfare legislation of Turkey and the European Union. The aim of the study is to give a first insight in the animal welfare situation at Turkish slaughterhouses. The investigations were carried out during a period of seven months (from May to November) in 99 slaughterhouses in 15 provinces of Turkey. The slaughterhouses were visited once and evaluated at the day of the visit using a standardised checklist which was designed according to the 'Council Directive 93/119/EC of 22 December 1993 on the protection of animals at the time of slaughter or killing' in order to be able to compare the monitored conditions in the different slaughter plants. The checklist was divided into six sections: general aspects, management and logistics, animal arrival, unloading facilities, lairage area, raceways towards slaughter room, restraining facilities and slaughter procedure; additionally a personnel performance index was used. The majority of all abattoirs provide basic unloading facilities like ramps, races and also a lairage area. The observed constructional deficiencies in numerous plants can be easily improved in most cases without causing high costs. As expected, the most significant difference to the European slaughter practice is the lack of any stunning prior to sticking and bleeding of the animals due to religious prescriptions which is not assessed in the framework of this investigation. The study reveals significant deficiencies in the education of the slaughterhouse staff. In future, special attention should be paid to the education of staff. Veterinarians and workers must be trained to detect weak and injured animals immediately and veterinarians must have the competence to make decisions on such animals whether they are fit for slaughter or have to be killed immediately followed by condemnation of the carcasses. A substantial welfare problem is that the animals are not sufficiently restrained. Consequently movements of the animal's body before and during slaughter cannot be avoided, what impairs fast exsanguination and leads to contact of the wound edges, causing pain. Appropriate restraint system should be applied in practice. The results of the investigation revealed a high variation between the quality of the slaughterhouses. On the other hand there is a high willingness to upgrade slaughterhouse conditions and to improve knowledge and skills of the staff. All the people involved were aware of the importance of animal welfare.

### **Multi-stage gas stunning of broilers in transport containers with carbon dioxide in two phases**

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Stunning poultry before slaughter is performed to secure animal welfare. In most slaughterhouses animals are electri-

cally stunned using a multi-bird water bath. To perform electrical stunning, animals are hung upside down in shackles while fully conscious, which is a major animal welfare issue in the stunning process. In order to minimise animal welfare problems due to handling at removal from the transport containers, a multi-stage gas-stunning system has been developed by which poultry are stunned with CO<sub>2</sub> while in the transport containers that are placed in a closed cabinet with controlled atmosphere. Basis of this approach is induction of unconsciousness with less than 40% CO<sub>2</sub> (Phase 1) followed by an irreversible stun using 65% CO<sub>2</sub> (Phase 2). In both phases CO<sub>2</sub> concentration is built up in stages to alleviate animal welfare problems and uncontrolled muscle contractions. Two different CO<sub>2</sub> recipes were investigated with 2 different exposure times. Both recipes allowed an increase to the Phase 1 CO<sub>2</sub> concentration (less than 40% CO<sub>2</sub>) within two stages. During recipe 1, the Phase 2 stunning-gas concentration (65% CO<sub>2</sub>) was built-up in five (5) stages with a total duration time of six (6) minutes. Recipe 2 comprised four (4) stages of one (1) minute each to reach the target Phase 2 stunning-gas concentration. Slow-wave EEG activity occurred at 36 (± 12) s with recipe 1 and at 29 (± 10) s with recipe 2. Suppressed EEG (loss of  $\alpha$  and  $\beta$  waves) occurred at 60 (± 23) s with recipe 1 and at 50 (± 24) s with recipe 2. Suppression of the EEG to approach iso-electric signal occurred at 4:07 (± 1:04) min with recipe 1 and at 3:27 m (± 50 s) with recipe 2. Slow heartbeat (40% of baseline bpm) occurred shortly after exposure to increasing CO<sub>2</sub> concentrations and prior to transitional EEG at 22 (± 13) s with recipe 1 and 29 (± 27) s with recipe 2. Heart failure occurs earlier with recipe 2 (4:04 [± 1:03] min) than recipe 1 (5:54 [± 1:39] min). No birds survived these experiments. The multi-stage gas-stunning system is a consistently functioning system in which poultry can be stunned with minimal risk to animal welfare. Animals were stunned within accepted EU regulations, ie CO<sub>2</sub> concentration during the induction phase remained below 40%.

### **2011 update of the 'AVMA Guidelines on Euthanasia' and creation of the 'AVMA Guidelines for the Mass Depopulation of animals'**

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Since 1963, the AVMA has convened a panel on euthanasia to evaluate methods and potential methods of euthanasia for the purpose of creating guidelines for veterinarians who carry out or oversee the euthanasia of animals. More than 70 individuals, including veterinarians and non-veterinarians with expertise across a range of disciplines and species, were engaged to research and create the 2011 update to the Panel's report (its eighth edition) titled the

*AVMA Guidelines on Euthanasia*. Euthanasia techniques should result in rapid loss of consciousness followed by cardiac or respiratory arrest and the ultimate loss of brain function. In evaluating methods of euthanasia, the panel used the following criteria: (i) ability to induce loss of consciousness and death with a minimum of pain distress, anxiety or apprehension; (ii) time required to induce loss of consciousness; (iii) reliability; (iv) safety of personnel; (v) irreversibility; (vi) compatibility with requirement and purpose; (vii) emotional effect on observers or operators; (viii) compatibility with subsequent evaluation, examination, or use of tissue; (ix) drug availability and human abuse potential; (x) compatibility with species, age, and health status; (xi) ability to maintain equipment in proper working order; (xii) safety for predators/scavengers should the carcass be consumed; (xiii) legal requirements; and (xiv) environmental impacts of methods or carcass disposition. The various sections of the Guidelines address particular euthanasia techniques (eg inhalant agents, non-inhalant pharmaceutical agents, and physical methods) and the application of those techniques to various animal types, species, and uses (eg companion animals, food animals, laboratory animals, wildlife, aquatics). This edition of the Guidelines has been expanded and includes more detail about the techniques, covers more species, and considers more comprehensively the special needs and challenges posed by the range of environments and conditions under which euthanasia is conducted. One topic that was not included in previous iterations of the Guidelines was methods for mass depopulation. Mass depopulation is the rapid destruction of large numbers of animals as a necessary response to animal health emergencies (eg control of infectious diseases) or natural disasters that place animals in exigent situations. Mass depopulation approaches may use euthanasia techniques, but not all mass depopulation methods meet the definition of euthanasia. For this reason, the AVMA has created a separate document titled *AVMA Guidelines for the Mass Depopulation of Animals*. This presentation will summarise the creation and content of both documents.

### **Electro-stunning of Atlantic salmon (*Salmo salar*): factors that affect the stunning current**

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The electrical parameters which normally define the efficiency of electrical stunning are voltage, frequency and current measured in ampere. For different types of animals and different animal sizes, there are different recommendations for the restricted intervals of these parameters. For

electro-stunning used in the farmed fish industry, a lot of efforts are taken to find the optimal combination values for voltage and frequency. However, little is done to clarify the ampere values' impact on the stunning efficiency. Research done on other animals, especially birds, has shown that there is a correlation between the bodyweight and the ampere on electro-stunning. The hypothesis was therefore that at a given voltage level there is a correlation between ampere and fish weight during dry electro-stunning. Because of biological tissues' non-linear electrical characteristics, it was expected a different correlation between ampere and fish weight at different frequencies and voltage levels. To clarify the ampere correlations to fish weight at dry electro-stunning, six different experiments were carried out. The experiments were done by placing the salmon on a stainless steel plate with a stunning shoe (of a commercial type delivered by STANSAS) over its head before the electric current was turned on and ampere values were measured. The ampere values at four different voltage levels were tested at two different frequencies. The voltage and frequency applied were 50 V/200 Hz, 110 V/200 Hz, 170 V/200 Hz, and 220 V/50 Hz. The stunning signal at 200 Hz was provided by a commercial used STANSAS 01 stunning machine. To create the 50 Hz stunning signal a standard ring type transformer was used. The fish size varied from 0.5 to 10.0 kg, where five of the experiments were carried out at the slaughtering facilities of Grieg Stjernelaks. The experiments were carried out in the period November 2008-June 2009. The results from the experiments do not verify the hypothesis. This is a rather unexpected result since variance in fish weight gives a difference in biomass, which represents a recessive load in the stunning system. In fact, it seemed that the voltage level and time of year had more influence on the measured ampere values than the fish weight. From the result of these experiments it is not possible to give a recommendation for variation of ampere values related to fish weight. Therefore, the most important electrical parameters for electro-stunning of fish still are voltage and frequency.

### **Development of standard operating procedures for the slaughter of Australian cattle in overseas markets**

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In March 2010, an independent expert review of the animal welfare conditions for Australian cattle in Indonesia was commissioned by Meat & Livestock Australia and LiveCorp. Three critical aspects were identified by the expert panel as significantly influencing animal welfare. These were:

- 1) Animal management; including handling, nutrition and animal suitability;
- 2) Slaughter; including facilities and method of slaughter;

3) Animal welfare standards and their practical application. The animal welfare issues encountered through the study were generally considered best addressed by extending or modifying programs currently being undertaken by Meat & Livestock Australia and LiveCorp with the support of the Australian and Indonesian governments. The purpose of this project was to develop a Standard Operating Procedures (SOP) manual for the slaughter of Australian cattle in overseas markets. The SOPs include chapters that cover identified key points of the pre slaughter and slaughter management of Australian cattle. The chapters included but were not limited to:

#### **a) Introduction**

- i) What is a Standard Operating Procedure (SOP)?
- ii) Overview of the slaughter procedure and desired outcomes
- iii) OIE requirements

#### **b) Receival**

- i) The environment
- ii) Preparation for slaughter
- iii) Receival process and management

#### **c) Lairage**

- i) The environment
- ii) Animal requirements

#### **d) Handling**

- i) Cattle behaviour
- ii) Low stress handling
- e) **Restraint** - Mark 1 and 4 cattle restraining boxes
- f) **Stunning** - pre and post stunning management

#### **i) Electrical**

#### **ii) Mechanical**

#### **g) Slaughter** - pre and post slaughter management

#### **h) Monitoring and reviewing SOPs** - Maintaining currency and encouraging continuous improvement

The SOP manual was designed for abattoir managers and government officials, where Australian cattle are slaughtered in Indonesia and the Middle East.

### **Causes of cattle bruising during handling and transport in Namibia**

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There are numerous risks associated with cattle transport and handling which all have the potential to cause bruising and poor welfare. The variables which were identified as high risk factors and had a significant influence on the level of bruising under Namibian transport conditions include animal factors (breed type, age, sex, condition and subcuta-



neous fat cover), pre-transport handling (re-branding of animals), transport-related risks (loading density, and animals lying down during transit) as well as lairage factors (fit of truck floor to off-loading ramp, the way animals moved to holding pen, pen size and minimum temperatures). The preliminary data analysis using multivariate principle component analysis showed some associations between the variables and the percentage bruising seen in the consignments. The strongest association was found with animals slipping on disembarking where 14% of the variation was explained by the model. When reducing the number of variables in the model, lairage duration (11% variation explained by model) and time of mustering on farm (9% variation explained by the model) also showed an association with bruising. Other associations between variables included travel speed and the amount of gravel road covered (11%); the percentage of animals lying on arrival at the abattoir and the distance of gravel travelled (10%); as well as an association between loading density and animals slipping during disembarking (9%). In the cluster analysis of the categorical data the percentage bruising was categorised as follows: A < 10%; B = 10–19%; C = 20–29%; D > 30%. Variables which associated with bruising level A were lairage duration and the longer distances covered on gravel during transport. Bruising level B was associated closely with horns while level C was grouped with total number of animals per consignment and number of animals slipping on disembarking. The highest bruise level was associated with the way animals disembarked and how many animals slipped during this process as well as the way the animals moved to their designated holding pen. However, no single factor could be pin-pointed as the major driving force behind bruise levels and the overall impression is that these risks have a cumulative effect on bruising. The results of this study indicate that in the event of animals transported to slaughter in the central areas of Namibia, conditions surrounding transport are just as important as distance transported or journey duration. To minimise bruising and stress of the animals destined for slaughter the following are suggested: on a producer level, it is suggested that the mustering period be shortened and that no re-branding be allowed. It is also suggested that farmers adhere to the transport standards as developed by the designated authority but that particular care be taken during transport on gravel roads. At the point of disembarking, care should be taken with the speed at which the animals are made to disembark — although it should be noted that this may be difficult to manage as a large percentage of the animals slaughtered are *Bos indicus* or crosses and/or raised under free-range production systems where they have very little interaction with humans.

### Alternative electrical stunning methods

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Current legislation demands that all birds are immediately rendered unconscious at stunning and that they remain insensible until death ensues. The legal minimal current for an individual bird in the water bath is 100 mA. During a survey, large differences were observed between slaughterhouses in the settings for water-bath stunning parameters for broilers. Based on the observed differences in technical settings, and between-animal differences in impedance and in sensitivity, it is highly probable that large numbers of birds are inadequately stunned during current usage of the water-bath technique in slaughterhouses. It was hypothesised that the placement of electrodes in order to bypass the feet and legs would result in efficiency gains by utilising lower currents. Broilers are effectively stunned with a controlled current of 70 and 100 mA for 1 or 1.5 s using a water bath where the head of the broiler is immersed in the water as electrode and a steel electrode at the cloaca as opposite electrode. There are fewer incidences of blood splashes in both fillets and legs of carcasses head cloaca stunned compared to those stunned in a conventional water bath. The method is in development for application in practice. An alternative to whole-body electrical stunning is head-only stunning, where the stunning current only passes through the head of the animal. Head-only single-bird electrical stunning was evaluated using a cone-shaped restrainer in which the broilers were suspended by their feet. Broilers may be insensible and unconscious after head-only electrical stunning with pin-electrodes using a current of 190 ( $\pm$  30) mA for 0.5 s. A set current of 250 mA is recommended to overcome individual differences in resistance. To prevent recovery the stun should be followed by an immediate neck cut. Since carcass quality is only slightly compromised such equipment is being developed further for commercial use. Transcranial Magnetic Stimulation (TMS) is a recently developed non-invasive technique used in human psychiatry to treat depression with slowly repeated pulses to the frontal lobe or to induce seizures. A study was done to determine whether or not TMS with an adapted coil for broilers has potential for further development as a non-invasive stunning method for broilers. TMS of the brain is a potential alternative for use as a stunning method for broilers. However, more research is required before further development for application as a stunning method.

## Progress in the humane slaughter of fish

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During the last 10 years, best practice in fish slaughter has developed significantly. In about 2000, the UK salmon industry moved away from manual percussive stunning in favour of the more consistent automatic equipment. Then, in 2003, the UK trout industry started to migrate from killing in ice slurry towards in-water stunning. More recently, the Norwegian salmon industry abandoned carbon dioxide in favour of automated percussion or dry electric stunning. Within the last year parts of the Dutch eel and catfish industries have started to use dry electric stunning rather than killing in ice or salt, some sea bass growers have declared an intention to adopt in-water electric stunning rather than killing in ice and parts of the Vietnamese Pangasius industry are adopting electric stunning rather than live bleeding. This poster discusses this progress, and describes these three approaches to humane slaughter. Information on the in-water stunning is covered in most detail since this is the technology with the authors are closely linked.

## A new system to control the sticking of pigs – VisStick

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**Background:** Although pigs are anaesthetised before slaughter, it is the bleeding that actually causes death. Sticking of the pigs is carried out manually by an operator and there is a risk that a pig may not be stuck properly, with the result that it may still be alive at the scalding. This raises serious welfare issues, and the DMRI has therefore developed a vision-based automatic system to monitor that all pigs have been stuck before moving to the next stage of the process. **Experimental:** The system consists of a camera, a lamp providing an evenly illuminated background and a computer. The camera records pictures, and the programme detects whether blood is running from the carcass or not. If no blood is detected, an alarm is activated to alert the operator to ensure sticking of the pig. System approval tests were carried out by DMRI monitoring 250 non-stuck pigs randomly divided among 500 pigs slaughtered successively. The false positives (alarms for stuck pigs) and false negatives (no alarm for non-stuck pigs) were recorded. **Results:** In approval tests of five typical installations a detection range of 98 to 100% of unstuck pigs was achieved. False positive results occurred in a range from 0 to 0.64%. A false negative result occurs if the VisStick system does not detect a non-stuck pig. A false positive alarm occurs if VisStick gives an alarm even though the pig

was actually stuck. DMRI-approval criteria for installations are presently a minimum of 96.8% capture of unstuck pigs and maximum 0.2% false positive alarms. The VisStick system has been installed in all major slaughterhouses with high line speeds in Denmark for more than two years. VisStick is now marketed and sold by DMRI to several slaughterhouses in the EU and the USA. **Conclusion and perspectives:** This system brings new technology into the slaughterhouses improving animal welfare by ensuring that all pigs are stuck before scalding. Furthermore, it may be possible to develop the system to be used for other animal species including sheep and cattle.

## Risk factors for poor welfare, including health of horses transported for slaughter within the European Union

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In certain parts of mainland Europe the consumption of horsemeat is popular and the distance between the origin of the horses and the meat markets has created a trade involving long-distance transportation of large numbers of live horses. The companion paper (Marlin *et al* 2010) identified evidence of poor welfare in horses being transported long distances to slaughter, including severe lameness, injuries and disease, and a high level of non-compliance, including unfitness for transport, with EU Council Regulation (EC) No 1/2005 on the Protection of Animals during Transport. **Objective:** To investigate risk factors associated with poor welfare, including health, of horses being transported long distances within the EU to slaughter. **Methods:** In order to investigate these issues, data on transported horses were recorded at two assembly centres in Romania and at four abattoirs in Italy over a seven-month period in 2008 (March to September). **Results:** A total of 1,519 horses in 64 separate shipments were observed in Romania prior to transport, of which, 212 horses were deemed unfit for transport in accordance with EU Council Regulation (EC) No 1/2005. Only 3 shipments (5%) were

both horse and vehicle compliant. Factors associated with poor welfare, including poor health, identified amongst horses being transported over long distances for slaughter within the EU included: lack of compliance with EU Council Regulation (EC) No 1/2005 on the Protection of Animals during Transport, origin, destination, vehicle type (including specific wheel configuration), time of year, external thermal environmental conditions (at the time of loading or unloading), the number of animals in the shipment, the stocking density and whether a veterinary surgeon was present during loading. **Conclusion:** In conclusion, risk factors for poor welfare, including poor health, amongst horses being transported over long distances for slaughter within the EU include: lack of compliance with EU Council Regulation (EC) No 1/2005 on the Protection of Animals during Transport, origin, destination, vehicle type (including specific wheel configuration), time of year, external thermal environmental conditions (at the time of loading or unloading), the number in the shipment, the stocking density and whether a veterinary surgeon was present during loading. **Potential relevance:** Horses being transported long distances for slaughter within the EU are at a high risk of poor welfare. This is due to a combination of the conditions under which these animals are maintained, loaded and subsequently transported and failure to comply with current EU legislation.

### **Slaughter of farmed salmon in Norway**

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The welfare of fish has become an explicit goal in legislation regarding fish farming and slaughter. One of the demands is that farmed fish shall be unconscious when bled. Some stunning methods are explicitly banned, among them carbon dioxide (CO<sub>2</sub>). However, this has been by far the most common method in Norway. In order to give the industry time to adapt, the ban has yet to be put into force. On behalf of the Norwegian fish farmers' organisation (FHL) we have evaluated the alternative methods of electricity and percussive stunning and their challenges and advantages under practical slaughter. Results from evaluations as well as research projects will be presented. Some important welfare challenges at slaughter are independent of stunning method. These include crowding of fish, pumping, poor water quality, and time kept out of water. These factors cause stress resulting in the depletion of energy reserves, and even physical injury. High fish flow rates may reduce the performance of the stunning equipment. A method for electrical stunning of fish submersed in seawater failed to satisfy the legal claim of

immediate unconsciousness. To avoid spine fractures and blood spots in the fillet, which may be a problem with electric stunning, this system made use of a pre-stun treatment with low voltage electrical stimulation, under which the fish were not rendered unconscious. Dry electric stunning, ie stunning out of water, has undergone a development with increasing voltage and improved stunning quality. A new device has been developed which orientates the fish head first into the stunner. This resolves one important concern addressed by EFSA. Also, the frequency of fillet quality problems has been reduced. However, there is lack of knowledge of risk factors for spine fractures and haemorrhages. There are two producers of percussive stunning equipment on the Norwegian market. Both stunners function well when used according to the producers' instructions. A correct stun causes irreversible unconsciousness or death. Variation in fish size is the main challenge. Also this equipment has steadily improved. The described alternative methods have been under continuous development the last few years and from a fish welfare point of view they are far better methods than CO<sub>2</sub>, which is highly aversive to fish. When CO<sub>2</sub> is used at low concentrations in combination with live chilling, the fish are sedated but not anaesthetised. Nitrogen gas does not seem to be suitable. There are promising preliminary tests on CO, a gas which does not seem to cause aversive reactions in salmon.

### **Stun assurance monitor (SAM)**

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The SAM is an innovative piece of equipment which offers exciting progress in the industry's ability to demonstrate high levels of performance in the stunning operation. In summary, the SAM provides: **Animal welfare:** The performance of the stun operation is of critical importance at the point of slaughter. The SAM provides a 'real time' monitoring capability for the stunner operator, abattoir management and the Official Veterinary Surgeon (OVS). **Stun operator training:** The comprehensive reporting facility within the PC software supplied with the SAM allows detailed analysis of operator performance. This can make a significant contribution to enable tailored training requirements to be constructed to increase performance standards. **Health & safety:** Analysis of each operators stunning performance and team comparisons can be used to identify fatigue and stress factors, increasing the ability to structure shifts and workloads to minimise risk to operators. **Tong performance:** Analysis by individual tong performance allows the early detection of faults, increasing the ability to maintain performance and avoid operational failure and throughput delays. **Quality control:** The impact of stunning on meat quality can be significant. The analysis of stun profiles can pin-point operational issues and allow corrective action to be taken, avoiding impact on carcass

quality. **Product provenance:** The use of SAM will enhance the provenance of resulting products through an increased ability to assure customers throughout the supply chain that animals were correctly stunned at this critical point within the slaughter process. **Halal:** The Muslim religion will only allow healthy animals to be slaughtered for halal. The ability to demonstrate that stuns are consistently delivered within agreed parameters will allow Muslims to accept stunning as a pre-requisite to slaughter. SAM confirms that animals were unconscious at the time of slaughter but not killed by the delivery of the electrical stun.

### **A major environmental cause of shipping fever in horses transported by air?**

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Transportation of horses by air has increased in volume since the first such flight was reported some 80 years ago. Despite the obvious health, welfare and economic concerns associated with the air transport process there has been surprisingly little research specifically aimed at the provision of scientifically based detailed guidelines and current procedures represent 'accepted best practice'. It is widely recognised that all modes of equine transportation may induce 'shipping fever' or bacterial pleuropneumonia and that the incidence and severity of the condition may be linked to the air quality experienced by the horses in transit. Thoroughbred horses are frequently transported by air over large distances and such journeys may be associated with 'shipping fever'. Detailed analysis of the hygrothermal transport micro-environment has not been reported. In particular, no information relating to absolute humidity is available. In the present study, therefore, 2 commercial flights (A) Sydney to New York and (B) Shannon to Sydney carrying groups of horses (A) 24 and (B) 11 were monitored to characterise the variability in the thermal micro-environment around and within jet stalls. On each aircraft, 20 data loggers, distributed throughout the transport space, were employed to record the air temperature and relative humidity at 1-min intervals. The flights were of 19 (A) and 25 (B) h duration. The mean temperature conditions on the two flights were: A) 20.9 ( $\pm$  3.8) $^{\circ}$ C, range: 13.6–20.9 $^{\circ}$ C and B) 19.9 ( $\pm$  1.1) $^{\circ}$ C, range: 15.1–25.4 $^{\circ}$ C. The corresponding mean water vapour densities were: A) 6.6 ( $\pm$  3.3) gm<sup>-3</sup> and B) 5.4 ( $\pm$  3.0) gm<sup>-3</sup> but with minima of A) 4.8 and B) 1.0 gm<sup>-3</sup>. The lower VD on the longer flight B was associated with post transport pathology in 7 of the 11 horses. Comparison of the water content of the air during the flights with air at sea level and calculation of the water vapour density gradients available for evaporation between the upper respiratory tract and inspired air indicates a signifi-

cant predisposition towards elevated drying rates of respiratory tract and associated dysfunction. It is proposed that a major predisposing factor to bacterial pleuropneumonia is the hygrothermal conditions prevailing in aircraft during long haul equine transportation.

### **Humane capture and slaughter of commercially caught wild fish: an emerging issue**

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The magnitude of a welfare problem may be quantified as the product of severity, duration and number of animals affected. In the case of commercially caught wild fish, the severity of suffering can be high, the duration extended and the numbers huge. Considerable suffering is likely to be caused to fish during capture, landing and subsequent processing. Fish probably experience fear, pain and distress as they are, for example:

- pursued to exhaustion by nets;
- crushed under the weight of other fish in trawl nets;
- raised from deep water and suffer decompression effects, eg burst swim bladders;
- snared in gill nets;
- confined in constricted seine nets;
- spiked with hooks (gaffed) to land them;
- caught on hooks, often for hours or days;
- impaled live on hooks as bait.

In many types of fishing the duration of capture can be very long, lasting hours or even days. Fish often die, or are fatally injured, during this process. Once landed, most fish are either left to asphyxiate, or die during further processing which may include gutting, filleting and freezing while alive and conscious. A small minority are slaughtered by the potentially humane methods of spiking the brain or percussive stunning. The current author has estimated the number of fish landed globally each year as 0.97–2.7 trillion, or in the order of a trillion individuals, based on FAO fisheries statistics and estimated mean weights for fish species (excluding shellfish, bycatch and illegal fishing). It is concluded that accepting that fish can experience pain and distress during commercial fishing is a major welfare issue. It follows that the welfare of wild-caught fish could be improved through measures to reduce severity, duration or numbers such as:

- using methods of capture and types of net/hook that reduce injury to fish;
- reducing the duration of the capture process;
- reducing stress and injury during landing;
- avoiding the use of live bait fish;
- humane slaughter as soon as possible after landing, including the adaptation of aquaculture technology;
- reducing the numbers caught through a range of fish conservation measures.



There would be economic consequences. Shorter duration times may increase labour and reduce catch sizes. Humane slaughter would probably require additional staff to manage the stunning and killing process. However, there are likely to be fish-eating and welfare-quality benefits which some consumers would appreciate the opportunity to pay for.

### **Basis for a tool for transporters to self-control the welfare of livestock during transport**

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Because of the growing importance of welfare issues regarding societal expectations, particularly in transport of farm animals to slaughter, new tools are needed to face all challenges of this issue. One of these challenges is for professionals to be able to self-assess the welfare of transported animals to identify and remedy difficulties. This study aimed to elaborate an operating tool dedicated to transporters for self-controlling the welfare of adult cattle, calves and sheep during transport. It was realised with the collaboration of the French professional organisations involved in livestock transport. Welfare indicators were first reviewed from recent international scientific literature. From this review, indicators were selected upon scientific relevancy and were tested by 3 observers in various transport contexts. Only indicators that were directly observable and measurable in any transport situation without impeding loading or unloading or without unloading animals when the truck is stopped were kept. Risks factors that can have a negative impact on the welfare of animals during transport were also listed. Several criteria were then defined to assess the welfare of animals at each step of the transport, to qualify loading and unloading areas, and to qualify handling during loading or unloading. Animal-based criteria related to the number of transported animals, to their fitness to transport and to their behaviour. These criteria differed between adult cattle, calves and sheep. Area criteria described loading and unloading areas or equipments. Handling criteria qualified the handler behaviour and the use of handling tools. Loading and unloading time was also recorded. All criteria were tested for reproducibility (Kappa index) within observers. Two complementary tools have been proposed to transporters:

- A handbook to describe each criteria and its measurement, with reminders of current regulation and good practices recommendations towards welfare issues;
- A set of 3 grids to observe and to qualify animal welfare, loading and unloading areas and handling. Different grids were proposed for adult cattle, calves and sheep.

These prototypes are now to be tested for feasibility by transporters, including drivers. They are a first basis for an operating tool dedicated to professionals for self-controlling the quality of their work towards animal welfare and then to answer the current society request on this topic.

### **Reduction of slaughterhouse stress in beef cattle by facilitating animal tameness**

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The relationship between animals and humans is important for animal husbandry and welfare. Loose-housing and grazing systems with low management input often result in frail relationships between humans and animals. This study investigated whether a positive handling, applied during the first days of the animals' life, had a calming and stress reducing effect on suckler beef calves at slaughter. Twenty-seven calves (male:  $n = 12$ , female:  $n = 15$ ) were assigned to a handled group (HG:  $n = 13$ ) or to a control group (CG:  $n = 14$ ), balanced for sex and age. Handling treatment, including elements of the TTouch©-Method, was conducted by a person being unfamiliar to the animals. Handling started the second day after birth and was continued on the third and fourth day of animal's life. Additional handling sessions were performed on three non-consecutive days during the following 3 weeks. Every session lasted 10 min and was repeated after 30 min. Handling treatments were always conducted in the home pen of the animal. At the age of 10 months, the not-yet-weaned animals were slaughtered at an abattoir in 30 km distance to the farm. Cattle transporters were loaded at the home pen with  $< 8$  animals familiar with each other. They were kept within their familiar group until stunning. The routine captive-bolt method was used for stunning. Animals were observed in the single-file race (order of entrance, prodding, going backwards, vocalisation) and during detention in the stunning box (duration of detention, head position in front of stunning operator, vocalisation). After slaughter, steaks from the musculus longissimus dorsi were analysed after 21 days of ageing for cooking loss (after 1 h cooking at 72°C), texture (Warner-Bratzler shear force analyses) and colour of the meat surface directly after cutting and after blooming for 1 h ( $L^*a^*b^*$  values). The two-sided Mann-Whitney  $U$  test was used for comparison of the means. The HG animals showed less avoidance behaviour towards the stunning operator (head position in front of stunning operator) than CG animals ( $P < 0.01$ ). Furthermore, prevalence of HG animals being the first ones to enter the single-file race was higher ( $P < 0.05$ ). Meat from the HG animals had a lower shear force than that of CG animals ( $P < 0.05$ ). We suggest that handled animals were less fearful towards humans at the abattoir and therefore showed less stress-related behaviour. This caused meat to get more tender. We conclude that actively facilitated animal tameness could be an approach to improve welfare and meat quality of cattle.

**Anaesthesia of broodstock Mediterranean fish**

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In aquaculture, there are many instances requiring some form of sedation or anaesthesia. Fish anaesthesia can be applied in fish transport, to immobilise fish during handling. In fisheries and aquaculture, there are many instances requiring some form of sedation or anaesthesia, such as live transport. Data concerning the use of anaesthetics in marine broodstock fish is almost inexistent. The aim of the present work was to provide information on the lowest effective dose for adult fish of four commonly used anaesthetic agents. Broodstock of Senegal sole (*Solea senegalensis*), gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) were used. The four anaesthetic agents tested were: Aqui-S®, iso-eugenol (50%) (Scan Aqua AS Årnes, Norway), 2-phenoxyethanol (100%) (Scharlab Chemie SA, Barcelona, Spain), and tricaine methanesulfonate (MS-222), 3-aminobenzoate methanesulphonic acid salt (100%) (Acros Organics, New Jersey USA). The fish were caught from their holding tanks and placed individually into an aerated anaesthetic bath (45 L), containing the same water as the holding fish tank. Four doses were tested per anaesthetic. Six or eight fish were individually tested per species. Oxygen, pH and temperature were recorded. For seabream and seabass, previously described stages of anaesthesia were assessed during sedation and recovery. For sole a new stage of sedation and recovery was developed. Each fish was observed timing each stage until it was anaesthetised. Afterwards, fish were transferred into a tank containing clean water and recovery was timed. Water quality parameters were kept constant for each species during all tests. Only MS-222 seems an acceptable anaesthetic for seabream, though recovery can reach almost 10 min. MS222 worked well for all species, doses required for sole are lower than for bream and bass. Aqui-S® seemed to be adequate only for sole, where even at the lowest concentration tested it induced a fast anaesthesia and recovery. 2-fenoxyethanol worked well for sole and seabass at 300 ppm, with recovery periods of less than 5 min and induction around 3 min.

**Nociception and pain in fish: implications for practices in the aquaculture chain**

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Consumer awareness and the interest of retailers for the welfare of farmed animals, including fish, is increasing. Consequently, more research is carried out to further our knowledge on fish welfare in aquaculture, including transport and (pre) slaughter methods. Scientific evidence for nociception in fish as in other vertebrates is accruing. Similarities in anatomy and functioning of the nervous system among all vertebrates are demonstrated. It has also been shown that fish possess an explicit memory, and thus the necessary modalities for pain perception and awareness are present in fish. Our ultrastructural analysis has demonstrated the presence of A- $\delta$  and C-fibres in tailfin clips of common carp (*Cyprinus carpio*), fibres known to transmit nociceptive signals in vertebrates. In a first experiment, we defined a series of physiological (branchial mucus release and content and chloride cell migration) and behavioural parameters (light/dark preference, swimming activity). These parameters were affected by a well controlled and supposedly painful stimulus (a standardised tailfin clip) to Nile tilapia (*Oreochromis niloticus*); responses were followed over a period of 24 h. In a second experiment, we investigated the effect of a standardised electric shock (15 Volt DC, 64 [ $\pm$  34] mA) applied at the tailfin of Mozambique tilapia (*Oreochromis mossambicus*) for 1 s. Again, we observed clear pain-specific effects of the stimulus on a set of parameters (plasma glucose levels, behaviour: activity and stereotypical behaviour performance), although the electric stimulus was less harmful than the clip. The stimuli investigated derive from aquaculture practices: indeed, fins are clipped to mark fish, but fins are also easily damaged during fights, handling, sorting, and transports. The electrical stimulus was chosen as, the European Food Safety Authority reported on stunning and killing of fish that these animals can be exposed to currents that are too low to provoke immediate loss of consciousness. Our results reveal that practices in aquaculture that lead to damage of fins or expose fish to electric shocks without immediate loss of consciousness should be avoided.

### **The pros and cons of various on-farm culling techniques**

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This Defra-funded project will examine the welfare implications of existing and novel on-farm culling methods for small numbers of poultry that are feasible to use on commercial poultry units, eg are portable, simple to use with minimal training, and require little equipment. Currently, the most common and simple method is manual cervical dislocation, because it requires no equipment and is relatively easy to learn. Cervical dislocation severs the vertebral column from the cranium and ruptures the blood vessels that supply the brain. However, loss of consciousness may not be instantaneous, which has serious welfare implications. Alternative, mechanical techniques include the 'Armadillo' where a spike penetrates and destroys the brain stem, but no scientific research has been undertaken to assess its effectiveness, and devices which are designed to stretch and twist the neck, such as Semark pliers or Burdizzo, but these may not sever the carotid arteries but merely crush the neck instead, for which reason they are banned as a method of stunning in the EU from 2013. There are also pneumatic or cartridge-powered percussive devices that apply a blow to the head, resulting in the immediate loss of consciousness and, when applied correctly, death. Although possible to shoot turkeys unrestrained, all other poultry require restraint to allow accurate positioning of the gun. This becomes increasingly difficult for smaller birds and ones which are highly mobile. In practical terms, both pneumatic and cartridge-powered devices have implications for cost, logistics/convenience and health and safety implications for operatives. In FAWC's 2009 recommendation, they expressed a desire for further refinement and development of such methods. We will review current and novel on-farm culling methods, in order to select methodologies to test during the project. We will then develop a robust and validated description of the visible signs and reflexes that are associated with brain indicators of loss of consciousness and death. Both the reviewed methods and some preliminary data on indicators of death will be presented at the Symposium. These visible signs and reflexes plus brain state indicators will then be used to assess how quickly the various methods induce loss of consciousness and death. Next, we will test the best methods under field conditions, using various bird types and different operators. Finally, we will collaborate with HSA to update guidelines on the best practice for killing poultry, including mitigation strategies where necessary.

### **Physiological monitoring of laying hens during whole-house killing with carbon dioxide gas**

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The ongoing threat posed by virulent avian influenza necessitates the availability of rapid, practical, cost-effective and humane methods of emergency killing for poultry. Whole-house carbon dioxide (CO<sub>2</sub>) administration has been utilised for this purpose, but there are no detailed studies of its welfare implications. We measured the physiological responses of ten hens equipped with sensors to measure body temperature, respiration, cardiac and brain activity (electroencephalogram, EEG) placed in a commercial poultry house undergoing application of CO<sub>2</sub>. Purpose-built telemetric logging units worn by each bird were used to record simultaneously these signals before, during and after gas exposure. Infrared video and thermography, air temperature and gas concentrations were recorded. Liquid CO<sub>2</sub> was injected into a deep pit, tiered cage, commercial poultry house via the pit and a gaseous concentration of 45% CO<sub>2</sub> was achieved within 19 min. Gas dispersal throughout the building was not uniform, with injection pressure initially increasing CO<sub>2</sub> concentration away from the injection site. Respiratory, cardiac and EEG responses and localised temperature in instrumented birds were related to position and hence CO<sub>2</sub> concentration. Although sub-zero temperatures were recorded in the immediate vicinity of a few birds, head, body and cloacal temperature measurements indicated no significant hypothermia. EEG characteristics were used to determine an unequivocal time to loss of consciousness. This ranged from 6.0 to 10.5 (average 7.8) min after onset of gas injection and was associated with CO<sub>2</sub> concentrations of approximately 20%. Birds did not experience the gas immediately so the period in which they were potentially conscious and exposed to increasing CO<sub>2</sub> was actually 3.9 to 7.9 (average 5.2) min. Distinctive cardiac and respiratory responses to CO<sub>2</sub> were seen, which were most marked in the conscious phase. In particular, birds responded to inhalation of CO<sub>2</sub> by deep breathing. Time to death varied between 13.7 and 22.1 min after gas injection and related to longitudinal position in the house relative to gas injection. Whole-house killing of hens using CO<sub>2</sub> is reliable and practical, and while birds exhibit prolonged reflex respiratory responses, they do not experience high concentrations of CO<sub>2</sub> while conscious and there is no evidence that they die of hypothermia.



### **Free-bullet rifle shooting of outdoor cattle**

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**Introduction:** Cow-calf herds for beef production kept outside year round have become more important in the context of species' appropriate livestock husbandry as well as landscape management in certain nature conservation areas. These types of management styles often lead to less frequent human-animal interactions. Animals not familiar with humans can be more challenging to handle, especially on the day of slaughter. Free-ranged animals without habituation may get heavily strained by stress factors such as spatial tightness, fodder deprivation, or close contact to foreign animals. The negative impact on meat quality due to pre-mortal stressors is known, eg dark-firm-dry meat (DFD). The purpose of this project is to enhance a possibility for cattle to get slaughtered without any dread. In addition to the animal welfare aspect, an economic surplus for the farmer could be achieved if there was a measurable improvement in meat quality. Thus, the first investigation focuses on the question of whether meat quality of beef cattle slaughtered on pasture differs from meat quality of ordinarily slaughtered animals from the same herd. **Animals, Material, Methods (Results:** summer 2011): In compliance with the German, as well as the EU regulation on the protection of animals at the time of killing, free-bullet rifle shooting is an allowable stunning method for outdoor cattle. Within the current investigation, 20 Galloways (2 per week) got slaughtered on pasture. For high shooting accuracy, the rifleman was situated as close as possible to the feeding herd (2–15 m). All herd members have been inspected the day before to enable free-shooting choice. Common signs of life were controlled immediately after shooting, eg no eye-ball rotation, no corneal reflex. A front loader placed the stunned animal into a special trailer which is considered a decentralised part of any EU-certified slaughterhouse. In this trailer, the killing by de-bleeding via breast-stick took place. Afterwards, the transportation to an abattoir for gutting and further processing started. Twenty Galloways of the same herd and age are slaughtered ordinarily, ie separated, caught, transported to the abattoir, lairaged, stunned by captive bolt and de-bled via breast stick. Investigation of meat pH and temperature 1, 24 and 48 h post mortem, tenderness, water-holding capacity and bleeding degree as well as glucose and lactate in blood. Gaining additional information via behavioural observation and pathological investigation of brain damage. **Following project (2012):** Investigation of stun quality of cattle stunned by free-bullet rifle shooting. **Acknowledgements:** We would like to thank for scientific co-operation A Sundrum, B Algers, M von Wenzlawowicz, M Bruegmann, G Kaemmer, L Trampenau and S Lindauer. We are grateful for funding provided by Karl Ludwig Schweisfurth, the Schweisfurth Stiftung, and the Bundesverband Deutscher Galloway-Zuechter eV.

### **Recent developments in euthanasia equipment**

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Accles and Shelvoke Ltd have been manufacturing 'CASH' Captive Bolt Humane Killers for nearly 100 years, and the equipment is well renowned and used throughout the world. The Cash Dispatch Kit is a recent development that has proven to be very effective in providing single-step euthanasia for the casualty industry, on commercial farms, companies transporting animals, mass euthanasia programmes and many other casualty situations. The kit includes a heavy duty Cash Special captive-bolt device with interchangeable bolt assemblies and different strength blank cartridges to provide a euthanasia system for a wide range of animals. The Cash Special on which the kit is based was introduced in 1980, replacing the world-renowned Supercash pistol. With substantial improvements in materials and ballistic design the Cash Special offers performance, quality and robustness never previously seen in the market place making it the most widely used and versatile captive-bolt tool in the world. Further developments of the Cash Special using heavier duty parts, improvements in materials and design allowing the use of more powerful blank cartridges has led to the development of the Cash Dispatch Kit. The product is available with penetrating and non-penetrating captive-bolt assemblies and a selection of cartridge strengths for different sizes and weights of animal. For poultry, newborn, suckling and small nursery pigs, lambs, goats and newborn calves, the kit provides controlled blunt-force trauma through the application of a specially designed non-penetrating captive-bolt assembly. The critical parameters for the bolt assembly include a muzzle face that is designed to accommodate the dimensions of the skull, a flat muzzle head with radiused edges and a restricted stroke length. Due to differences in critical physiological parameters (skull thickness, bone hardness, depth of brain, etc) of various species and age of animal a system of muzzle/bolt assemblies and developed forces is necessary to provide single step euthanasia capabilities. Therefore a variety of penetrative bolts and a selection of cartridge strengths are available in the kit. A penetrative short-bolt assembly is suitable for lambs, nursery and grower pigs. A medium length bolt assembly is designed for larger pigs, sheep and average-size cattle and finally, a long-bolt assembly can be selected for boars and sows, heavy cattle and bulls and horses. The tool is provided with a detailed reference guide ensuring easy-bolt assembly and cartridge selection, and each bolt assembly is marked with the maximum strength cartridge that can be used. Following extensive research and trials, the effectiveness and flexibility of the Cash Dispatch Kit has already proved itself as providing a single-step euthanasia system for the livestock industries.



### **Delivering nitrogen gas to poultry sheds for emergency killing using high expansion foam**

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Whole-house gassing (WHG) is used for emergency killing of poultry flocks on farms in the event of a notifiable disease outbreak. However, not all poultry buildings are suitable due to the fact they cannot be adequately sealed, in particular free-range poultry houses. A system was developed by LST International BV which generates high expansion foam containing nitrogen gas instead of atmospheric air. This foam can be deployed directly into sheds without prior sealing. Experiments on individual birds had determined that the birds died by exposure to the gas itself not the foam. It was observed that wing flapping of poultry might cause destruction of the foam and so the height of foam above birds before flapping onset (15–18 s after submersion) was critical. A series of experiments were undertaken to determine the parameters required of the system for deployment into poultry sheds. In trials conducted as the system was developed, it was determined that it could produce stable foam to height of at least 3.7 m and flow down a 15 m shed at a height of 2 m. In final experiments prior to evaluation with birds, the foam-generating system consisted of a bulk tank of liquid nitrogen connected to a fan-assisted ambient air vaporiser and an integrated system consisting of a water tank, foam concentrate tank, pump and electro-mechanical proportioning system to supply the generators at the required pressure and with the correct concentration of foam solution. For the experiments, four individual foam generators were connected to the system and positioned at one end of a pen. Gas-foam with an expansion ratio of 340–50:1 was delivered from the generators at a combined rate of 42.5 m<sup>3</sup> min<sup>-1</sup>. The foam bow wave flowed down the shed at a rate of 0.15 ms<sup>-1</sup>, which would ensure that individual birds were submerged with 50 cm within 5 s of contact by the foam. The average time taken to reach a depth of 60 cm was 11 s and 30 s to reach 1 m. In order to achieve a consistent bow wave the foam should be generated across the whole width of the shed, rather than a single point, so that it reaches the sides and builds height as quickly as possible. Measurement of oxygen concentrations within the foam showed that it was below 1%. The results allowed a design standard and application protocol, including operating procedure and monitoring points to be produced.

### **More Danish cattle correctly euthanised after information campaign**

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Mortality among Danish dairy cows has tripled during the last two decades, from approximately 2% in 1990 to 6% in 2010. Mortality includes both cows dying unassisted and cows being euthanised on-farm. Previous research has shown that part of the increase in cow mortality is caused by an increasing number of euthanised cows over time. This increase in euthanasia is mainly caused by a changed threshold for euthanasia among Danish dairy farmers. Economic and legal changes have caused farmers to lower their threshold for euthanasia. This may have a positive impact on animal welfare as more seriously ill cows are euthanised at an early stage of the disease. However, euthanasia must be performed correctly and without suffering for the cow. Approximately 75% of all cows euthanised in Denmark are shot (penetrating captive bolt) by the farmer or a veterinarian and 25% are euthanised by an overdose of an anaesthetic. According to Danish legislation, exsanguination (bleeding) after shooting is mandatory. Exsanguination subsequent to shooting is needed to ensure the death of the animal and failure to exsanguinate may result in severe suffering for the animal. Failure to exsanguinate thus constitutes a problem both legally and in relation to animal welfare. All dead cattle in Denmark are processed at one incineration plant. To evaluate the proportion of shot cows and calves which had been correctly exsanguinated, a random sample of 196 shot cows and 196 shot calves was evaluated at the incineration plant in 2004. We found that only 24% of cows and 4% of calves was exsanguinated after shooting. Based on these very low numbers of correctly euthanised cows and calves an information campaign was launched by the Danish Cattle Federation, the Danish Veterinary Association and the Danish Animal Welfare Society. The aim of the campaign was to inform farmers and veterinarians about the need to exsanguinate shot animals. To evaluate the effect of this campaign, we repeated the evaluation at the incineration plant in 2010. This time 199 cows and 212 calves was evaluated and we found that 91% of shot cows and 61% of shot calves had been exsanguinated. Most of the calves not exsanguinated were male Jersey calves. Significantly more animals had been correctly euthanised in 2010 compared to 2004 and the information campaign seems to have had a good effect. However, there is still 'room for improvement', especially in relation to correct euthanasia of calves.

### **Automated online scoring of foot-pad dermatitis in broiler chickens at the slaughterline: evaluation and correspondence with subjective expert scoring**

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Broiler slaughterhouses give feedback to the farmers about the status of the birds that have arrived. This makes it possible to evaluate the management of a farm at the slaughterhouse. In the past, the EU was thinking about linking a specific welfare parameter that is easily measured at the slaughterhouse, to the welfare of the animals at that specific farm. One of the possible parameters was the foot-pad dermatitis score. But this idea was abandoned in the end and one reason was that no objective scoring systems existed. Now, an automated system was developed that makes it possible to score foot-pad dermatitis at the slaughter line. In this article the first prototype was evaluated. Now, a second prototype exists and is being evaluated. The MEYN system for the automatic scoring of foot-pad dermatitis (FP) in broiler chickens at the slaughterhouse was compared with the subjective scores given by an expert both on-farm (OF) and at-slaughter (AS). Broilers (40 d) from an experimental farm were scored for FP by the expert until about 20 animals were identified per FP score (0-4, classification according to Welfare Quality®). Subsequently, these batches of 5 × 20 broilers were scored again on FP at the slaughterhouse first by the MEYN system (meyn) and then by the same expert (blind to the result of the previous scores). This experiment was repeated once. The pictures and image processing algorithms that were taken by the MEYN system were collected and evaluated for obvious errors. Twelve and 18% of the broilers were not scored by Meyn in rounds 1 and 2, respectively. For 48% (round 1) and 30% (round 2) of the broilers, the Meyn system recognised and scored both foot pads. The wrong side (backside) of a foot was scored twice (2%), and a neighbouring foot pad was erroneously scored three times (3%). There was a strong correlation between the subjective OF and AS scores ( $r = 0.83$ ), but the correlation between OF and meyn ( $r = 0.54$ ) and between AS and meyn ( $r = 0.59$ ) were weak. With the Meyn system there was a significantly higher chance for a higher FP score as compared to the subjective OF (odds = 2.32) and AS (odds = 2.46) scores. The MEYN system allows FP condition to be scored automatically without slowing down the slaughter speed, but improvement of the system is still needed and was recently established. The second prototype is now under evaluation by ASG from The Netherlands and ILVO.

### **Main outcomes and recommendations for good animal welfare practices during religious slaughter**

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A specific objective of the DIALREL European Community project 'Religious slaughter: improving knowledge and expertise through dialogue and debate on issues of welfare, legislation and socio-economic aspects' was to propose recommendations for improving animal welfare during religious slaughter, including the restraining, neck cutting and post-cut management, taking into account the existing legislation and religious slaughter requirements. As reversible stunning is accepted by some religious communities, recommendations for pre- and post-slaughter stunning were also considered. Restraint (design, construction, operation and maintenance) has a marked impact on animal stress that will, in turn, impact on the quality of the cut, bleeding and the time to loss of consciousness. In cattle, the use of an upright pen can reduce the duration of restraint before neck cutting and it allows the animal to be slaughtered in a natural standing position. However, this position may require greater skill to achieve an appropriate cut and manage the post-cut period. Restraint in a rotary pen can facilitate neck cutting. However, inverted restraint may lead to increased stress. Dorsal recumbency (animal turned on the back) is an unnatural posture and might also cause discomfort. Turning to positions between upright and lateral recumbency (eg 45 or 90°) has the potential to decrease stress. Incising the neck can result in noxious stimuli that will be perceived as pain in conscious animals. However, the issue is controversial, as there are differences in cutting method and variation in the time to loss of brain function between reported studies. In addition, wounds or actions that involve scraping of exposed tissues and large or multiple cuts, are more likely to elicit pain. If an animal is not stunned before slaughter, it becomes unconscious when brain perfusion declines after the neck cut. The time taken for unconsciousness to supervene varies between cattle. Post-cut stunning shortens the time to unconsciousness, ie the time when the animal can feel anxiety, distress and/or pain. Recommendations have been developed in dialogue with stakeholders, how animal welfare during religious slaughter can be improved in practice.

### **Evaluating short (under 8 h) transport journeys of sheep in the UK in terms of welfare plus environmental and economic consequences**

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The UK has approximately 33 million sheep and has evolved a unique system of production which makes efficient use of pasture on highland, upland and lowland. This results in a complex supply chain with numerous, predominantly short (under 8 h), journeys between different types of pasture and to slaughter. The journeys vary considerably, from a simple movement of a single group of animals from one point to another, to a trip involving multiple pick-ups and a stop at a market or collection point before arrival at the final destination. The aim of this study is to quantify the number and different types of short journeys which sheep may undergo during marketing in the UK. It will not only measure the impact of these differing types of journey and marketing system on the welfare of the sheep, but will also concurrently evaluate the environmental and economic consequences of different short journey types for sheep. Results are not yet available, but the approaches are outlined as follows: i) to measure welfare, data will be collected on the behaviour and physiology of 1,500 sheep finishing their 150 journeys at slaughterhouses. Analysis will indicate the relative effects of journey variables such as live auction markets, multiple pick-ups, journey duration, weather, age, sex, breed, etc on the welfare outcomes; ii) the frequency with which the different journey types occur will be determined from records of all sheep movements held in the Animal Movement Licensing System (AMLS2). With supplementary information, this will produce a summary of the movements occurring in the UK to place the welfare measurements into context; and iii) a conceptual framework of sheep transport and market systems will be developed which identifies:

- the stakeholders in the transport process;
- the different purposes of movements;
- points where financial costs are incurred;
- the resulting environmental emissions and;
- the resources that are consumed.

Using this, together with data from AMLS2 and follow-up interviews with hauliers and auctioneers to establish additional costs, it should be possible to determine the costs of transporting sheep over different journey lengths and with different vehicle types. Finally, the information will be combined to allow the costs and benefits to animal welfare of the differing parts of supply chains to be compared with the environmental and economic costs and benefits. **Acknowledgement:** This study is supported by Defra (AW0943).

### **Abnormal behaviour in horses transported long distances across Europe for slaughter: preliminary findings**

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Observations made by World Horse Welfare suggest that abnormal behaviour and behaviour indicative of poor welfare (ie pain, stress, exhaustion, etc) are common amongst horses transported long distances across Europe for slaughter. A field study was undertaken to collect preliminary observational data relating to the behaviour of horses during a 24-h off-the-vehicle rest period at an Italian control post. Observations took place in September 2010 during daylight hours. Behavioural data were collected from a total of 18 horses from one randomly selected shipment; horses were observed individually during two-minute observation periods. Behaviour considered to be abnormal or indicative of poor welfare was observed in all horses. This behaviour included: abnormal stance suggestive of pain or discomfort (94%; n = 17); weight shifting (83%; n = 15); depressed or withdrawn demeanour (39%; n = 7); licking and chewing (33%; n = 6); low head carriage (28%; n = 5); licking and mouthing objects (28%; n = 5); swaying (22%; n = 4); and flexing hind limbs (17%; n = 3). Hay was available to all horses but only seven horses (39%) were observed foraging or eating. Water was provided periodically, but horses did not have access to water during data collection. No horses were observed urinating, defaecating, vocalising or mutual grooming. There was one display of inter-horse aggression. General observations were recorded from a further 77 horses from four separate shipments. These observations revealed additional frequently occurring or repetitive behaviours, including: resting/placing one or both forelimbs on or in the water trough; attempting to climb into the water trough; lip flapping; pawing (particularly stallions); and chin resting. No horses had constant access to water but all horses observed during watering were seen to drink. All horses displayed signs indicative of thirst including: aggression towards other horses when watering commenced; drinking despite apparent fear of the hosepipe; extended drinking bouts and a voracious appetite for water (eg subsequent analysis of footage from static mounted cameras revealed one uninterrupted drinking bout lasting in excess of five minutes). In general, horses drank until the water trough was empty. It is suggested, in light of these findings, that the absence of obvious signs of disease or injury is not sufficient to deem a horse fit for transport and that behavioural indicators should be included in any welfare assessment. This preliminary study highlights the need for further investigation into the incidence, aetiology and prevention of abnormal and stress-related behaviours in horses transported long distances across Europe for slaughter.

### **Traumatic brain injury associated with captive-bolt euthanasia of swine**

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In the USA, captive-bolt technology is acceptable for euthanasia of swine when followed by exsanguination or pithing. A new generation of captive bolt technology (Cash Euthanizer, Accles & Shelvoke Ltd, UK) is designed for single-step euthanasia, with 4 interchangeable heads and gun-powder cartridges specific each weight class of pig. The objectives of this study were: (i) to assess the effectiveness of the Cash Euthanizer for single-step euthanasia of seven weight classes of pig; (ii) to assess traumatic brain injury associated with captive-bolt euthanasia. Forty-two anaesthetised pigs, 3 males and 3 females from each of 7 weight classes (2–3 kg, 7.5–10 kg, 15–20 kg, 30–40 kg, 100–120 kg, 200–250 kg, > 300 kg) were euthanised using the Cash Euthanizer. A non-penetrating head was used for the 2 lightest classes, and 3 bolt lengths corresponded to the 5 heavier classes. Duration of movement was used to assess the clonic phase. Death was determined by

cessation of cardiac activity, and a 10-min ceiling was used after which a secondary euthanasia method (electrocution) was applied. Heads were removed and fixed in formalin for two weeks before dissection. Traumatic brain injury (TBI) and haemorrhage scores were collected for the cerebral cortex, thalamus, cerebellum, pons and medulla regions. Data were analysed using a one-way analysis of variance, and pair-wise *t*-tests were used to explore differences between the 7 weight classes. There was a trend for difference between classes need of a secondary methods of euthanasia ( $P = 0.0951$ ), for 4 animals in the 2 heaviest classes. None of these pigs displayed clonic movements. On average, clonic movements ceased 1.7 min following firing of the captive bolt pistol and there were no differences between weight classes ( $P = 0.6990$ ). On average, cessation of heartbeat occurred 3.9 min after firing the of the captive bolt pistol, with no differences between weight classes ( $P = 0.3188$ ). Cerebral cortex TBI scores differed by weight class ( $P = 0.0068$ ), with TBI scores of mature sows and boars differing from the scores for farrowing, nursery and grower pigs. The cerebral cortex is associated with interpretation of sensory information, and hence is significant for assessing quality of euthanasia. The thalamus is associated with wakefulness and consciousness. Traumatic brain injury scores for the thalamus differed between weight classes ( $P < 0.0001$ ); damage was not observed in 3 largest classes. In conclusion, our data supports captive-bolt technology as a single-step method of euthanasia for swine under 200 kg. Factors, such as placement, may be associated with inconsistent results observed for mature pigs, and warrants further study.