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Pre-slaughter conditions, animal stress and welfare: current status and possible future research

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The present paper describes the main procedures used to slaughter fowl, pigs, calves and adult cattle, sheep, and farmed fish, starting on the farm and ending with the death of the animal at the abattoir. It reviews the currently known causes of stress, indicated by behavioural and physiological measurements on the animal level, and by post-mortem muscle metabolism. During the pre-slaughter period, psychological stress is due to changes of environment, social disturbances and handling, and physical stress is due to food deprivation, climatic conditions, fatigue, and sometimes pain. The exact causes of stress depend, however, on the characteristics of each species, including the rearing system. For fowl, bird catching and crating, duration and climatic conditions of transport and of lairage and shackling are the main known pre-slaughter stress factors. For pigs, stress is caused by fighting during mixing of pens, loading and unloading conditions, and introduction in the restrainer. Handling and novelty of the situation contribute to the stress reactions. For veal calves and adult cattle, disruption of the social group, handling, loading and sometimes unloading conditions, fatigue, novelty of the situation and for calves mixing with unfamiliar animals are known stress factors. Gathering and yarding of extensively reared lambs and sheep causes stress, particularly when shepherd dogs are used. Subsequent transport may induce fatigue, especially if sheep are commercialised through auctions or markets. In farmed fish, stress is predominantly related to environmental aspects such as temperature, oxygen, cleanliness of the water and, to a certain extent, stocking density and removal of the fish from the water. If transport and lairage conditions are good and their durations not too long, they may allow pigs, calves and adult cattle, sheep, and fish to rest. For certain species, it was shown that genetic origin and earlier experience influence reactions to the slaughter procedure. Stunning techniques used depend on the species. Pigs and fowl are mostly electrically or gas-stunned, while most adult cattle are stunned with a captive bolt pistol. Calves and sheep may be electrically stunned or with a captive bolt pistol. Various stunning methods exist for the different farmed fish species. Potential causes of stress associated with the different stunning procedures are discussed. The paper addresses further consequences for meat quality and possible itineraries for future research. For all species, and most urgently for fish, more knowledge is needed on stunning and killing techniques, including gas-stunning techniques, to protect welfare.

Keywords: behaviour, muscle metabolism, physiology, slaughter, stress

Introduction

Since the publication of Ruth Harrison's book Animal Machines in 1964, public opinion with regard to animal welfare has much changed. Animal welfare is considered a physical and mental state related to the absence of negative emotions (Dawkins, 1990; Duncan, 1996) and probably to the presence of positive emotions (Boissy *et al*., 2007). The term of stress refers to the behavioural, physiological and emotional status of the animal confronted with a situation that it perceives as threatening with respect to the correct functioning of its bodily or mental state (Désiré *et al*., 2004; Terlouw, 2005). Much effort has been made to reduce stress, and thus to improve animal welfare during the rearing period. Today, animal protection during the pre-slaughter and slaughter period receives increasing attention. In line with this, the Council directive

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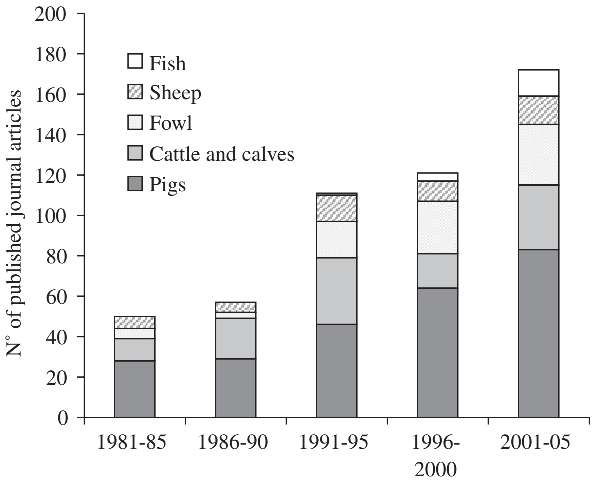

Figure 1 Number of journal articles published in English in international journals, containing the key-words 'stress' and 'slaughter' anywhere in the text, as retrieved by 'web of science' (isiknowledge.com) over the past 25 years.

93/199/EC from December 1993 points out that 'Animals shall be spared any avoidable excitement, pain or suffering during movement, lairaging, restraint, stunning, slaughter or killing'. Over the past 25 years, research on the effects of slaughter conditions on animal stress and ante- and post-mortem muscle metabolism has progressively increased (Figure 1). Many papers focus primarily on the consequences of meat quality, but more and more specifically study animal welfare matters, or at least discuss them.

Slaughter procedures and therefore the possible causes of stress during this period vary according to species. Stress at slaughter may be of physical origin. Long and rough transports, food deprivation, inappropriate temperatures or air or water quality, aggression or shocks may cause fatigue, hunger, thermal and respiratory discomfort and pain, respectively. It may also have a psychological origin, such as social disturbance (separation from the rearing group, mixing of unfamiliar animals, high density) or fear (unfamiliar environments, handling). The slaughter procedure is complex and often a situation may represent several potential stress factors. For example, transport is associated with a change in physical and social environment, movements of the lorry that may cause physical shocks and unfavourable climatic conditions (temperature, humidity and air drafts), or water quality deterioration for fish. European standards have laid down maximal transport durations of 8 h for adult animals of all land species discussed here. For longer durations, the animals should be given a rest period and liquid and, if necessary, food (EU Directive 95/29/EC of 29 June 1995 and Council Regulation (EC) No. 1/2005 of 22 December 2004; Von Borell and Schäffer, 2005; Cockram, 2007). It is further mandatory for slaughterhouses to provide food to animals that have not been slaughtered within 12 h of their arrival (Council Directive 93/199/EC).

Stunning before slaughter is a statutory requirement in Europe and is performed to induce unconsciousness and insensibility in animals so that shackling, hoisting and slaughter can be performed without causing the animals any avoidable anxiety, pain, suffering or distress. If animals are slaughtered without previous stunning for religious/cultural reasons, the abattoir needs a special licence. The stunning technique is part of the slaughter procedure and the type used depends on the species (Table 1). Electrical stunning is induced by an electrical current passing through the brain resulting in a brain status similar to a grand mal epileptic fit due to a substantial depolarisation of the neurons. This status is considered incompatible with normal neuronal function and therefore believed to induce unconsciousness until repolarisation of the neurons (Hoenderken *et al.*, 1979; Cook *et al.*, 1992 and 1995). Mechanical stunning most often involves the introduction of a captive bolt into the skull. It provokes, first, an impact causing shock waves and subsequently destroys part of the brain resulting in a loss of consciousness. If the bolt is correctly introduced, loss of consciousness is permanent, although the bolt does not, in itself, kill the animal (Daly *et al.*, 1987 and 1988; Finnie, 1993). For gas stunning, a reversible technique unless the animal is killed, animals are immerged in gas mixtures containing CO_2 and air, or argon and nitrogen (Raj *et al.*, 1997; Gerritzen *et al.*, 2000). Fish may, in addition, be stunned by exposure to cold temperatures (icy water). After stunning, the animals are killed by bleeding using neck cutting or chest-sticking techniques. Neck cutting can be used for most land species and implies the severing of the major blood vessels in the neck, where skin and vessels are cut simultaneously. Chest sticking, used for mammals, implies the severing of major blood vessels in the thorax or chest by inserting a knife in front of the brisket or sternum. In the case of cattle, chest-sticking needs more time, because first the skin, then, with another knife, the vessels are cut. Fish are bled by gill arch section or by manual pulling out of the gills, or by caudal vein

Table 1 *Stunning methods used for different species*

Species of animals	Generally used stunning methods in Europe
Cattle and calves	Mechanical Electrical[1]
Sheep	Mechanical Electrical[1]
Pigs	Mechanical Electrical[1] Gas mixtures[1]
Poultry	Electrical[1] Mechanical[2] Gas mixtures[1]
Fish[3]	Manual/Mechanical (percussion[1], spiking[2]) Thermal Electrical[1] CO_2

After Anon. (2004).
[1] May induce death.
[2] Induces death.
[3] Sometimes fish are left out of the water before bleeding, slowly inducing unconsciousness. Sometimes, they are bled without stunning.

cut, or by decapitation. Smaller fish species are not always bled. For all species, death due to blood loss should be induced before return of consciousness. All these stunning techniques are often preceded by physical and psychological discomfort for the animal. Animals may be isolated, constrained, inhale unpleasant gases, be subjected to temperature changes or shackled. In any case, proper use of stunning methods needs the presence of trained operators and adequately maintained equipment (Grandin, 2006).

To assess stress responses at slaughter, in addition to classically used (neuro)physiological and behavioural measurements, assessments of immunological status and ante- and post-mortem muscle metabolism have shown to be very useful. Data on resting behaviour, slips and escape attempts give useful information on the way the animal perceives its environment (Lensink *et al.*, 2001). The effects of physical activity and psychological stress on the secretion of hormones, measured in blood, urine or saliva and heart rate are well known and can be used to assess stress at slaughter (Foury *et al.*, 2005; Hambrecht *et al.*, 2005; Terlouw and Rybarczyk, 2008). Stimulus-induced electro-encephalocortical responses allow one to assess whether unconsciousness has been correctly induced by the stunning technique (Raj *et al.*, 1992; Robb *et al.*, 2000; Martoft *et al.*, 2002). If genetic and environmental (rearing/carcass handling) aspects are controlled for, post-mortem muscle metabolism reflects partly pre-slaughter stress and physical effort (Debut *et al.*, 2005; Terlouw, 2005). Following slaughter, glycogen continues to be degraded, but in the absence of blood circulation, protons and lactate accumulate locally in the muscle cell, causing a decline in pH (Bendall, 1973). This decline is initially fast, then slows and stabilises at a value called ultimate pH, reached 24 h *post mortem* (usually between 5.4 and 6.0 for land animals and higher than 6.0 for fish). High muscle temperature and a fast pH decline during the minutes or hours following slaughter indicate increased activity and possibly psychological stress just before slaughter. A high ultimate pH (low amplitude of pH decline) indicates that the animal had reduced glycogen reserves, probably due to increased activity and possibly psychological stress during the hours preceding slaughter (Morzel *et al.*, 2003; Terlouw, 2005). However, pH may evolve normally, even if an animal has been stressed before slaughter (Terlouw and Rybarczyk, 2008).

The following chapters briefly describe the main procedures used to slaughter the following most consumed species: fowl, pigs, calves and adult cattle, sheep and farmed fish, and discuss the currently known associated causes of stress. We address subsequently consequences on meat quality and possible itineraries for future research.

Fowl

Fowl are either reared in very large groups of several thousands on a floor system (broiler chickens and turkeys for example) or in cages containing only a few animals (layer poultry birds for example). At least 8 h before slaughter, animals are food deprived. Before transport they need to be caught to be introduced into transport crates that are loaded onto a lorry. European legislation has fixed that transport durations over 8 h should be avoided and that food and water should be made available for transports lasting over 12 h. European standards for density during transport range from 180 to 105 cm^2/kg for weights ranging from <1.6 to >5 kg live weight. At arrival at the slaughter house, the transport crates may remain for a while on the lorry until unloading, outside or in a shed. Depending on the time schedule of the abattoir and the time of arrival, they may be rapidly slaughtered or wait for several hours. The birds remain inside the crates. For slaughter, a conveyer belt takes the crates towards the stunning area. Here the birds are manually suspended on their legs on shackles that run automatically along a rail towards an electrified water bath. The birds receive an electrical shock when the head immerses in the bath, causing loss of consciousness. The birds are bled immediately after the water bath. In some European countries, birds may be gas-stunned, using various mixtures of CO_2 with Argon and/or Nitrogen, or CO_2 with O_2. In this case, the cages are put onto a conveyer belt that enters the gas area.

Food deprivation of the birds before slaughter may have negative effects on animal welfare. Two hours of food deprivation is accompanied by an increase of corticosterone, which may result from metabolic stress and also have a psychological component (Kannan and Mench, 1996; Nijdam *et al.*, 2005). After 24 h, the birds may lose up to 10% of their initial weight (Warriss *et al.*, 1999). Food deprivation reduces hepatic and muscle glycogen reserves, which may increase the birds' reactivity to stress factors during transport and slaughter (Sams and Mills, 1993, Kotula and Wang, 1994; Edwards *et al.*, 1999).

Bird catching is probably a major cause of stress during the pre-slaughter procedure. The birds are carried upside down by their legs and introduced in transport cages, which may cause bruising and dislocated and broken bones (Gregory and Wilkins, 1989; Kettlewell and Mitchell, 1994). In broilers, carrying the birds upside down caused an increase in plasma corticosterone (Kannan and Mench, 1996). During subsequent crating, corticosterone levels remained high, unless the crated birds were kept in a dark, quiet place (Kannan and Mench, 1996; Kannan *et al.*, 1997b). Some automatic bird-catching machines exist. They cause less fear reactions, as indicated by lower levels of tonic immobility, plasma corticosterone and lesions compared to manual catching (Duncan *et al.*, 1986; Knierim and Gocke, 2003). They are, however, little used.

The welfare of the birds depends strongly on the duration and conditions of transport and waiting on the unloading dock at the slaughterhouse. For example, longer transports increased fear and stress reactions as indicated by higher levels of tonic immobility, of plasma corticosterone and creatine kinase and the heterophil/lymphocyte ratio, as well as the number of animals found dead on arrival (Freeman *et al.*, 1984; Cashman *et al.*, 1989; Mitchell *et al.*, 1992; Warriss *et al.*, 1992). During bird transport, climatic micro-environments exist in the lorry. There is an accumulation of

CO_2 and heat, especially at the core of the vehicle load (Mitchell and Kettlewell, 1994) and particularly increased temperature is considered one of the sources of transport stress (Mitchell *et al*., 1992). Thermal stress during transport or during lairage caused increased plasma creatine kinase and corticosterone levels in birds (Mitchell *et al*., 1992; Mitchell and Sandercock, 1995; Debut *et al*., 2005). No water is available in the crates, and dehydration may aggravate the effects of thermal stress (Gregory, 1998; Knowles *et al*., 1995b). More birds are found dead on arrival after transports at higher temperatures (Gregory, 1994). Increased physical efforts of the food-deprived birds, during transport or during the waiting period at the slaughterhouse, explain their lower hepatic and muscle glycogen levels (Warriss *et al*., 1999).

The procedure for electrical stunning may have negative effects on the welfare of the birds. Shackling causes an increase in corticosterone, but has no effect on tonic immobility (Kannan *et al*., 1997a; Debut *et al*., 2005). The shackled birds vocalise and flap their wings probably with the objective to regain the upright position (Gregory and Bell, 1987; Debut *et al*., 2005). Mecanothermal C-fibre nociceptor responses to mechanical stimulation indicate that shackling may be very painful for the birds (Gentle and Tilston, 2000) and may cause broken bones, at least in layer hens (Gregory and Wilkins, 1990). Wing flapping was violent or prolonged when shackles were tight fitting (Parker *et al*., 1997). Processing plants may use dim light (<5 lux) or blue or violet light, which has a quietening effect on birds compared with light of 50 or 200 lux (Jones *et al*., 1998). Birds that perform more wing flapping on the shackle line have a faster pH decline, due to acceleration of their muscle metabolism just before death (Debut *et al*., 2005). Genetic background influences the degree of struggling during shackling with more struggling in a slow-growing compared to a standard fast-growing chicken line, possibly explained by anatomic differences (Debut *et al*., 2003).

Before entering the water bath, birds may receive pre-stun shocks, generally because their wings hang lower than their heads and touch the water first. Pre-shock stuns may induce wing flapping and consequently the head of the bird may partly or completely miss the water bath. Occurrence of pre-stun shocks is higher in turkeys than in chickens, due to the larger wing span of turkeys (Hewson and Russell, 1989; Wotton and Gregory, 1991). The problem is reduced if the water does not overflow at the entrance of the water bath and if the bath is fitted with an electrically isolated entry ramp that slopes upwards towards the bath. Their effect is to support the birds' heads until the end of the ramp where the head drops suddenly into the water bath (Wotton and Gregory, 1991).

Electrical water baths vary according to manufacture. They are all constant voltage (not constant current) stunners and most use frequencies between 300 and 1500 Hz of pulsed direct currents (DC) or sine wave alternating currents (AC). Minimal current needed to induce consciousness, and duration of induced unconsciousness depend on all of these characteristics (Raj and O'Callaghan, 2004), as well as on species of the birds. Commercial water baths may contain up to 20 chickens or five turkeys. Resistance depends on the number of birds at any one time in the water bath, i.e. its length, cleanliness of the water, conductivity between the shackles (earth electrode) and the birds' shanks and impedance of the birds (Schutt-Abraham *et al*., 1983; Bilgili, 1992; Sparrey *et al*., 1992). In the case of electrical stunning, rapid subsequent neck cutting is important. In larger plants, neck cutting is often automatic with a rotary blade severing at least one but preferably two carotids. Delay to neck cutting (i.e. the distance between the water bath and the cutting device) should be in accordance with minimal duration of unconsciousness induced and hence with electrical parameters used.

Gas stunning requires much less manipulation of the animals and avoids the pre-stunning shackling procedure, because the crates with the birds are directly put onto the conveyer belt that transports them into the gas room. The method is used in slaughterhouses in certain European countries. In the case of birds, the duration of exposure and gas concentrations are often conceived to kill, rather than only stun the birds, to avoid that the birds wake up while they are bled. Delay to cutting is not important, as the birds are dead. There are also some negative aspects to this method. Inhalation of some gases, such as CO_2, is unpleasant for humans: at higher concentrations, it is pungent and induces the sensation of breathlessness (Gregory *et al*., 1990). Various mixtures can be used to stun or kill poultry; e.g., 90% argon in air, 30% CO_2 and 60% argon in air, or 40% CO_2, 30% O_2 and 30% N_2 (Lambooij *et al*., 1999). In birds, certain mixtures may be aversive, as 30% of CO_2 in argon and 30% of CO_2 in 40% of O_2 (remaining part being air) induced gasping (Gerritzen *et al*., 2000). Immersion in high concentrations of argon might be a better, albeit a more expensive solution (Raj, 1996; Gerritzen *et al*., 2000). In addition, induction of unconsciousness takes longer, and recovery time is shorter when argon is used without CO_2 (Raj and Gregory, 1990 and 1993).

Other techniques, captive bolt pistol or neck dislocation, are only used as back-up stunning methods. Decapitation without prior stunning is only used for small-scale on-farm killing or for disease control. It induces loss of brain responsiveness ($<5\%$ of pre-decapitation spontaneous electrical brain activity) after 32 ± 2 s (Gregory and Wotton, 1986).

Pigs

Pigs are generally reared in groups of 10 to 12. Live weight of slaughter pigs is generally between 110 and 115 kg in most European countries. At arrival of the lorry, they are loaded and are either directly transported to the slaughterhouse, or they stop at other farms to fill the lorry. In the lorry, pens of a same rearing site are mixed. Sometimes, before arrival of the lorry, pigs of different pens have already been mixed while waiting on the loading quay of the farm. European legislation has fixed a maximal density of 0.51 m^2 per pig of 100 kg live weight during transport. In the slaughterhouse, pigs are lairaged in pens containing from 15 up to sometimes 50 pigs, depending on the slaughterhouse equipment. Depending on the time schedule of the abattoir and the time of arrival, they may be rapidly slaughtered or wait for 3 to 18 h. In general, pigs have often been food deprived for at least 12 h, but sometimes up to 24 h before slaughter. Pigs of different farms are not mixed. For slaughter, pigs are guided to a stunning box or restrainer for electrical stunning, or to a gas stunner.

Adequately designed and maintained equipment to load pigs onto the lorry avoids slipping of the animals and favours rapid introduction of the pigs in the lorry, reducing stress. Both loading and unloading procedures, even well organised, are associated with physical effort of the pigs and provoke increased heart rates and plasma levels of cortisol and creatine kinase (Geverink *et al.*, 1998; Kim *et al.*, 2004; Brown *et al.*, 2005). Three hours of transport required physical effort as it caused net glycogen consumption, sometimes resulting in higher ultimate pH (Pérez *et al.*, 2002; Hambrecht *et al.*, 2005). The effect of transport on physiological and behavioural parameters depends mostly on conditions. Hambrecht *et al.* (2005) observed that after 50 min and 3 h of transport cortisol responses were similar. If abrupt changes of speed and direction are avoided and if ambient temperature is adapted, the pigs are able to adopt a stable posture and even rest (Geverink *et al.*, 1998). If the lorry is in good condition, driving is smooth and stocking density appropriate, the stress and effort related to loading and unloading have probably the largest effect, possibly explaining the higher plasma cortisol levels and lower neutrophil/lymphocyte ratio after short (15 min) compared to longer (3 h) transport (Pérez *et al.*, 2002). However, the level of psychological stress due to social disturbances, including mixing of pigs, to handling and unfamiliarity of the situation, should also be considered and will be discussed below.

From the meat quality point of view, given that immediate slaughter after arrival at the abattoir may cause accelerated post-mortem pH decline, it is recommended to lairage pigs, for 1 or 2 h, but depending on the time of arrival, lairage may last up to 18 h. The consequences of lairage at the abattoir for the well-being of the pigs vary between studies and depend strongly on stocking conditions. A major cause of stress is the mixing of pigs of different pens (from the same farm), which results often in fighting. Fighting may start at the farm, if the pigs are mixed on the loading quay, or at the abattoir, during lairage. Fighting may occur during transport (Barton-Gade, 2004) if transport conditions (stocking density, driving conditions) allow for it. Fighting levels depend often on the presence of a few aggressive animals in the group (Geverink *et al.*, 1996) and are generally higher at higher stocking density (Geverink *et al.*, 1996), in larger compared to smaller groups (Schmolke *et al.*, 2004) and in pigs that have been food deprived (Brown *et al.*, 1999). The latter point shows that, although up to 12 h of food deprivation does not seem to cause physical discomfort (Beattie *et al.*, 2002), food deprivation increases reactivity to certain slaughter procedures and therefore contributes indirectly to stress levels at slaughter. Fighting increases plasma levels of cortisol, adrenaline and metabolites, and meat ultimate pH increases proportionally to fighting levels (Fernandez *et al.*, 1994; Terlouw *et al.*, 2005). However, if lairage groups are small and duration of food deprivation is short, the lairage period may allow pigs to rest and stress levels may progressively decrease (Perez *et al.*, 2002).

Following lairage, pigs are driven to the stunning area. Duration and conditions of this phase depend on the equipment and line speed of the abattoir. If the corridors contain corners or obstacles, if the entrance of the automatic electrical stunning system presents a bottleneck, or if groups are very large, pigs progress with more difficulty (Grandin, 2006). Use of sticks and electric prods obviously causes pain and stress (D'Souza *et al.*, 1998; Hemsworth *et al.*, 2002).

The pig's history influences reactivity to slaughter procedures. Pigs reared outdoors or in enriched environments fought less and had lower skin damage levels (Klont *et al.*, 2001; Barton-Gade, 2008; Terlouw *et al.*, 2004). It is believed that rearing at high density or under barren conditions may disturb the normal development of social behaviour or increase fearfulness, resulting in increased aggression (Schouten, 1986; de Jonge *et al.*, 1996; Olsson *et al.*, 1999; O'Connell *et al.*, 2004). Pigs reared under standard intensive conditions were easier to load, but showed a stronger increase in salivary cortisol during transport and lairage than pigs reared in larger pens or in an enriched environment and their meat had more drip loss (Geverink *et al.*, 1999; De Jong *et al.*, 2000; Klont *et al.*, 2001; Chaloupkova *et al.*, 2007), although opposite results have also been reported (Lambooij *et al.*, 2004).

The different methods used to stun pigs have different advantages and disadvantages. Electrical stunning can be induced very quickly, with manual stunning tongs or automatic systems. Automatic electrical stunning systems have the disadvantage to contain a restrainer that pigs must enter in a single line. The restrainer carries the pigs, either through two slightly inclined conveyer belts or on a central rail towards the stunning electrodes at the exit. These position themselves automatically behind the eyes to deliver the electrical shock. The restrainer causes a sharp rise in heart rate (Chevillon, 2001) probably because it contradicts the gregarious instinct of the pigs and because it lifts their feet from the floor. At the narrow entrance of the restrainer, pigs may mount on other pigs, injuring them. Sometimes, when the electrodes position themselves wrongly due to different sizes of pigs, or when maintenance is insufficient, the current applied may be insufficient and painful (Sparrey and Wotton, 1997; Wotton and O'Callaghan, 2002). Some more recent automatic electrical stunning systems apply a third electrode on the back or chest to cause cardiac fibrillation or arrest, to avoid that animals regain consciousness (Wotton and Gregory, 1986).

Carbon dioxide stunning is much used in some European countries. The system consists of a pit of several metres filled with a high (generally 70–90%) concentration of CO_2. CO_2 is heavier than air and therefore forms a gradient inside the pit, with lower concentrations at the upper levels. Pigs enter a box within the upper part of the pit, which subsequently closes and lowers into the pit. The system may be conceived for individual and grouped pigs. Today, there is no consensus on the level of aversion of the CO_2 stunning system. The possibility to keep the pigs in groups is clearly an advantage. As mentioned above, inhalation of high concentrations of CO_2 is unpleasant for humans (Gregory *et al*., 1990). Food-deprived pigs refuse to enter an area containing 90% of CO_2 to obtain a food reward (Raj and Gregory, 1995), while Jongman *et al*. (2000) demonstrated that inhaling 60% or 90% CO_2 is less stressful than receiving an electrical prod. Pigs inhaling 80% CO_2 loose their standing posture after about 22 s (Raj and Gregory, 1996). At lower CO_2 concentrations, loss of posture and consciousness takes longer (Dodman, 1977; Raj and Gregory, 1995 and 1996). Observations on 10 pigs found that immersion in 80% of CO_2 caused unusual behavioural excitation. Eight pigs responded with violent jumps, all of them had apparent respiratory difficulties and eight vocalised (cf. Deiss *et al*., 2006). There is currently no consensus on the question whether pigs lose consciousness before or after the behavioural signs (Hoenderken *et al*., 1979; Martoft *et al*., 2002).

Studies have been conducted with the aim to better understand underlying factors involved in the stress reactions of pigs at slaughter. Like other species, pigs are known to show consistency in their responsiveness to different stressful situations (Lawrence *et al*., 1991). Many of the slaughter procedures are associated with handling of the pigs and, therefore, reactivity to human presence was tested several weeks before slaughter, during the rearing period. Large White pigs that in the human exposure tests approached and touched more easily a person had lower muscle temperature after slaughter. This suggests that they had lower stress and/or activity levels just before slaughter (Terlouw and Rybarczyk, 2008). Other experiences show the opposite, probably because pigs that were less fearful of man were at the end of the queue and received more adverse interactions (Hemsworth *et al*., 2002). Genetic background plays a role in reactivity to humans: in the human exposure test, Duroc pigs approached less often the person than Large White pigs (Terlouw and Rybarczyk, 2008). Reactivity to novelty may also play a role in the stress response, as Large White and Duroc pigs that explored more a non-familiar object during a test conducted during the rearing period produced meat with higher ultimate pH, indicative of increased activity during the pre-slaughter period. Part of the increased pH was explained by the higher aggressiveness during mixing in these same pigs (Terlouw and Rybarczyk, 2008; Figure 2).

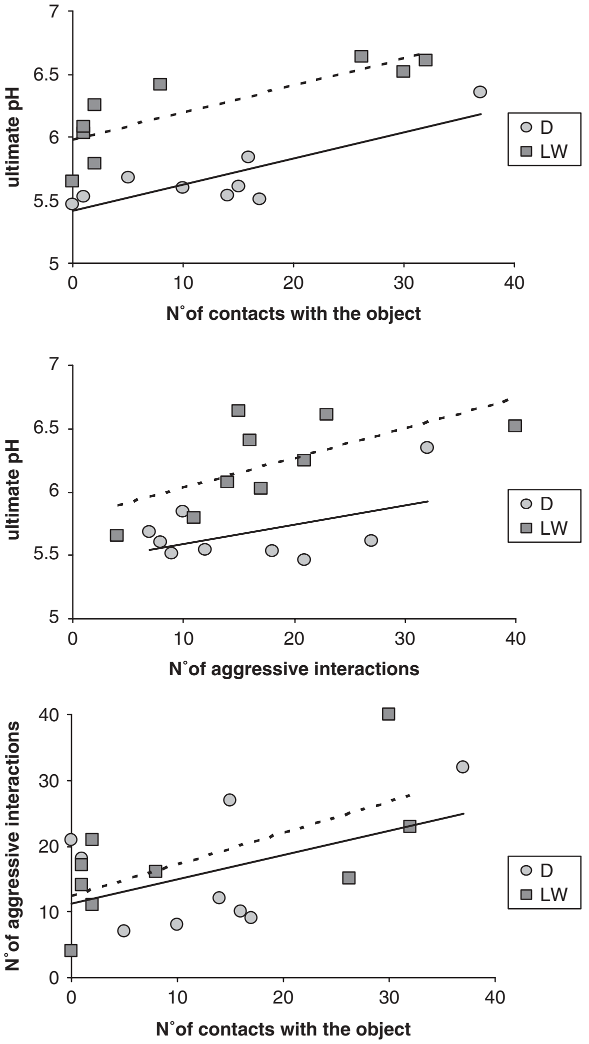

Figure 2 Scatter plots and regression lines between Adductor femoris ultimate pH (indicative of pre-slaughter stress reactions; see text) and bouts of exploration of the unfamiliar object (top; Pooled Pearson, $r = 0.83$; $P < 0.001$) and the number of aggressive interactions during mixing before slaughter (middle; Pooled Pearson, $r = 0.59$; $P < 0.01$) and between bouts of exploration of an unfamiliar object during a test and the number of aggressive interactions during mixing before slaughter (bottom; Pooled Pearson, $r = 0.57$; $P < 0.01$) for industrially slaughtered Duroc and Large White pigs. Data from Terlouw and Rybarczyk (2008).

Veal calves and adult cattle

Veal calves and fattening bulls are mostly loaded as large groups from a single farm. Cull cows and sometimes bull calves, as well as neonatal male calves in the UK where veal calf production is very low, are loaded individually. If only few animals are loaded per farm, the lorry is filled with animals of several farms and consequently cattle from different farms may be mixed in the same compartment. Cattle are slaughtered either immediately after arrival at the slaughterhouse or after a lairage period of 12 to 24 h. Today adult cattle are generally lairaged in individual stalls. Veal calves travel generally directly from the farm to the slaughterhouse, with transport durations between 1 and 4 h. They are lairaged in groups of 20 to 40 calves. The lairage period is generally kept short, between 30 min and 3 to 4 h, to avoid bruising resulting from the fact that the calves are tired and lie down. Stocking density standards during transport depend on the weight of the animal, from 0.4 m^2 for a calf (100 kg), up to 2 m^2 for an adult (1000 kg). Adult cattle are generally stunned with a captive bolt, although in some countries several abattoirs use an automatic electrical stunning system (Wotton *et al.*, 2000). Depending on the country, calves are electrically or captive bolt stunned (Lambooij and Spanjaard, 1982).

Of the transport procedure, the loading procedure on the farm seems the most stressful aspect for calves and adult cattle. The animals have to leave their familiar pen or environment and sometimes social group, are handled and are introduced into the unfamiliar environment of the lorry, sometimes containing unfamiliar animals. The whole loading procedure causes an increase in the blood cortisol rate and an increase in the heart rate of approximately 80% in calves, compared to home pen levels (Lensink *et al.*, 2001; Van de Water *et al.*, 2003). Loading conditions, especially equipment (loading ramp and quay), determine largely levels of psychological stress and physical effort. Despite efforts, farms are generally insufficiently equipped. Slippery floors, sharp corners or metal structures may hurt the animals and further cause psychological and physical stress (Lensink *et al.*, 2001; Van de Water *et al.*, 2003; Mounier *et al.*, 2006).

During transport, heart rate and plasma levels of cortisol, creatine kinase, free fatty acids and lactate are higher than those observed in the home pens (Van de Water *et al.*, 2003; Grigor *et al.*, 2004). Sartorelli *et al.* (1992) showed that cortisol levels increase during the first 30 to 60 min of transport and then remain stable. Heart rate is initially high, but decreases gradually over a period by 30 to 60 min of transport, reaching values of 10% to 25% above the basic level (Lensink *et al.*, 2001; Van de Water *et al.*, 2003; Grigor *et al.*, 2004). These physiological changes are most probably related to emotional stress due to the unfamiliar situation and to physical effort related to loading and to keep balance in the moving and vibrating lorry (cf. Van de Water *et al.*, 2003).

Transport conditions (temperature, draughts, driving style), the density and the position of the animals in the truck influence behavioural and physiological responses of calves (Kenny and Tarrant, 1987a and 1987b; Van de Water *et al.*, 2003). For example, calves at the rear of the lorry have higher heart rates and lower cortisol levels than those at the front (Van de Water *et al.*, 2003). Increased heart rate was explained by the increased effort to keep balance at the rear and increased cortisol levels by changes in the temperature and draught, inducing a physiological stress, at the front of the lorry (Van de Water *et al.*, 2003). During long transport at low density, calves may lie down, probably expressing fatigue (Grigor *et al.*, 2004). Although this allows them to rest, calves remaining standing may injure those lying down.

Introduction of adult cattle into the lorry caused an increase in frequency of urination and cortisol levels and these increased further when the truck started moving, to decrease after approximately two hours of transport (Tarrant, 1990). Adult cattle may lie down after 2 to 4 h of transport if conditions are adapted allowing the animals to rest (Broom, 2003), which is, however, rare. Adult cattle prefer to be oriented parallel or perpendicularly to the driving direction. Their orientation depends further on the driving style and road type (Kenny and Tarrant, 1987a and 1987b). Loss of balance and falling is likely to cause injury or crushing by conspecifics. Appropriate stocking density is important. High stocking density increases cortisol levels and makes it difficult for the cattle to orient themselves according to the driving direction. At too low stocking density, the animals cannot use surrounding animals to maintain their balance (Tarrant, 1990; Knowles, 1999). Balance maintenance in these large animals involves great physical effort and may cause fatigue (Tarrant, 1990). Such effort explains the increased levels of plasma creatine kinase after 15 h of transport (Knowles, 1999).

Several studies showed that the rearing system and the type and frequency of contacts with the stockperson influence the animals' fear reactions to novel situations during the pre-slaughter period. For example, the calves having received positive and more frequent contacts from their stockbreeder were easier to handle and had lower heart rates during loading (Lensink *et al.*, 2000a, 2000b and 2001). Keeping the animals in stable groups may further facilitate the slaughter procedure. Cattle tend to follow their conspecifics, thus reducing stress due to separation or emotional reactions to the slaughter context. Bull calves kept in their social group during the pre-slaughter period had less increase in cortisol levels in blood collected at bleeding (Mounier *et al.*, 2006). The effect was even more pronounced when the animals had been reared together (Mounier *et al.*, 2006). Other studies found that cattle differ consistently in their reactivity to various aversive situations and that this reactivity was correlated to reactions to slaughter procedures, indicated by differences in bruising score and tenderness (Fordyce *et al.*, 1988; Voisinet *et al.*, 1997).

At arrival at the slaughterhouse, animals are again exposed to various potential stressing factors such as handling, novelty and particularly for calves, mixing with unfamiliar conspecifics. Unloading conditions at the slaughterhouse are generally better than loading conditions at the farm, due to the better equipment. Although the lairage environment may be stressful for adult cattle, the lairage period may allow a recovery of the physical efforts and the psychological stress related to the unloading and transport procedure. After 24 to 48 h lairage of adult cattle, behavioural, physiological and metabolic status is similar to before transport (Warriss *et al.*, 1984; Knowles, 1999; Mounier *et al.*, 2006). In calves, heart rate during unloading shows similar increases as those observed during loading at the farm, i.e. 70 to 75% increases compared to basal levels (Lensink *et al.*, 2001). Cortisol levels, increased after transport, reach pre-transport levels after about 2 h of lairage, while heart rate levels remain higher, suggesting psychological stress (Lensink *et al.*, 2001; Van de Water *et al.*, 2003; Grigor *et al.*, 2004). At lairage durations of over 2 h, the calves tend to lie down due to fatigue, which increases risks of slips, falls and injury (Grigor *et al.*, 2004).

Mixing of unfamiliar adult cattle during transport or at lairage results in agonistic interactions. Young bull calves, in addition, mount each other, causing muscular fatigue and glycogen depletion (Kenny and Tarrant, 1987c). Mixing of adult cattle may thus have negative consequences for animal welfare and meat quality. Therefore, today, height of the stocking compartments of the lorries is limited to prevent mounting and lairage of adult cattle takes place in individual stalls.

Few data are available on the reactions of calves and adult cattle to the stunning procedure. The fear-inducing aspects of handling, certain odours, social isolation and darkness in the stunning box are well known (Terlouw *et al.*, 1998; Grandin, 2006). The use of the electric prod is painful and increases vocalisation, indicative of stress (Rushen *et al.*, 1999; Grandin, 2001). Penetrative captive bolt stunning, when correctly applied on the front of calves or adult cattle, has generally good results in terms of success rate and duration of unconsciousness. However, field observations indicate that 6.1% to 16.3% of miss-stuns may occur, often due to inadequate maintenance of the gun, or to bad quality of the cartridges (Von Wenzlawowitz, personal communication, 2004; Gregory *et al.*, 2007). Electrical stunning can be applied manually on young calves (Bager *et al.*, 1992). If the system is properly maintained and used, unconsciousness can be reliably induced. Manual electrical stunning may be used with very low throughput rates for adult cattle that are calm or restrained (Von Mickwitz *et al.*, 1989; Schatzmann and Jäggin-Schmucker, 2000). For less-calm animals or at higher throughput rates, automatic systems should be used.

Electrical stunning has also some disadvantages. Electrically induced unconsciousness may not last long enough to allow normal killing by bleeding if cardiac ventricular fibrillation has not been also induced by application of a current in the cardiac region. Even if fibrillation has been induced, the heart can resume normal functioning if an animal is manipulated too soon after application of this so-called stun/kill procedure (Daly, personal communication). Another negative aspect is that electrical stunning (whether associated with cardiac fibrillation or not) induces tonic/clonic seizures, making prompt and accurate sticking difficult (Wotton *et al.*, 2000). In some parts of the world, electro-immobilisation (low-voltage spinal discharge) is applied following the stun to prevent the movements. However, electro-immobilisation masks the signs of consciousness in poorly stunned animals making visual monitoring of undue recovery of unconsciousness inadequate (Wotton *et al.*, 2000; Anon, 2004). Alternative systems allowing electrical stunning and immediate sticking within the restraining pen before seizures may present advantages for animal welfare (Troeger, 2002), but are probably not adapted for higher line speeds.

After stunning, cattle are neck-cut or chest-stuck. In adult cattle and calves, delay of loss of consciousness after neck cutting is very variable and was observed to last up to 680 s (Bager *et al.*, 1992). After chest-sticking, mean arterial blood pressure fell from about 70 mmHg to 0 within 8 s, suggesting that loss of consciousness is much faster and less variable after this technique (Anil *et al.*, 1995b). The reason is that at neck cutting only the external jugulars and the common carotids are severed, but not the vertebral arteries that continue to supply blood to the brain. In addition, after neck cutting, blood clots appear sometimes at the caudal ends of the severed carotids, causing occlusions and delaying the onset of unconsciousness (Bager *et al.*, 1992; Anil *et al.*, 1995a and 1995b; Gregory *et al.*, 2006). The chest-sticking technique is thus more efficient and particularly useful when reversible stunning techniques are used. However, chest-sticking takes more time as it is preceded by a skin cut (see Introduction).

Sheep

Sheep are bred indoors or outdoors, sometimes in extensive systems. In many European countries, sheep bred for meat are mostly slaughtered as lambs, between 1 and 8 months of age (Sañudo *et al.*, 2007). Before transport to the slaughterhouse, outdoor reared sheep are gathered and yarded, sometimes with the help of a shepherd dog. The structures of journeys by which sheep travel to slaughter vary greatly. At simplest they are directly from the farm to the abattoir, but often they involve stops at a number of markets and farms en route. When animals are sold through electronic auctions, they are often widely scattered, making it hard to pick them up in one day. Such complex journeys often involve the separation of familiar sheep and/or the mixing with unfamiliar conspecifics and quite long transport durations. Accepted density by European standards depends on animal status (shorn/unshorn, pregnancy, weight), from 0.20 to $>0.50\,m^2$/animal. In the slaughterhouse, sheep are either directly slaughtered or lairaged overnight in groups of variable sizes. They may be washed before slaughter for hygiene, and, if they are electrically stunned, to improve stunning effectiveness due to better conductivity.

The collection of sheep in a waiting pen on the farm involves handling of the flock. Driving the flock by the shepherd provoked behavioural reactions, such as trotting and vocalisation and increases in heart rate and cortisol (Fordham *et al.*, 1989; Baldock and Sibly, 1990). Sheep little used to handling show stronger reactions (Bassett and Hinks, 1969). Use of a dog by the shepherd caused strong reactions, raising heart rates 3.5 times the pre-trial level (Baldock and Sibly, 1990) and cortisol levels 8 times (Durand *et al.*, submitted). Separation of sheep from their rearing group or mixing of different groups also caused behavioural reactions and increases in heart rate (Baldock and Sibly, 1990; Parrott *et al.*, 1994). The increases in heart rate were not fully explained by the increased activity of the animals, suggesting significant emotional stress (Baldock and Sibly, 1990).

At loading and during the first hours of the journey, heart rate also increased (Knowles *et al.*, 1995a; Broom *et al.*, 1996; Kent, 1997; Hall *et al.*, 1998), probably again indicating both effort and emotional stress of the animals. Plasma cortisol level of sheep increased strongly during the first 20 min and was still high after 24 h of transport (Parrott *et al.*, 1994; Cockram *et al.*, 1997). Behavioural observations suggest that sheep quieten down only after 9 h of transport at a density of 0.29 m^2/animal (38 kg) (Knowles *et al.*, 1995a). Posture depends much on space allowance and transport conditions. Cortisol increases less on smooth than rough journeys (Bradshaw *et al.*, 1996). Lambs (35 kg) at a density of 0.25 m^2/animal will lie down if travelling on smooth roads and may ruminate, although rumination is less than in confined, non-transported controls (Cockram *et al.*, 1996, 2000 and 2004). Prior experience may play a role as outdoor sheep lay down and ruminated less during transport (30–40 kg; 0.2 m^2/sheep) than those from inside pens (Cockram *et al.*, 2000). Sheep appear to physically support transport up to 24 h rather well. For example, increases in plasma creatine kinase observed during single journeys up to 24 h are considered minor (Kent, 1997). In addition, measurements of plasma osmolality, total plasma protein and albumin did not indicate severe dehydration of the sheep after 24 h of transport (Knowles *et al.*, 1995a; Broom *et al.*, 1996; Cockram *et al.*, 1996). However, body energy reserves were mobilised during a 24-h journey, as indicated by increases in free fatty acids and β-hydroxybutyrate levels and upon arrival, feeding and drinking activity was greater after than before transport (Knowles *et al.*, 1995a; Parrott *et al.*, 1997; Cockram *et al.*, 1997 and 1999). Breeds differed in their physiological reactions to transport (Hall *et al.*, 1998). The passage at distance markets ($>$500 km from the market place) is a source of additional stress, causing bruises and further raising plasma cortisol and creatine kinase levels and osmolality (Jarvis *et al.*, 1996).

While cold can generally be compensated by the heat produced by the animals, exposition to high temperatures increases mortality rate during transport (Gregory, 1998). Acceptable thermal conditions depend of the fleece length. For shorn ewes, the lower critical temperature is around 20°C and the upper 40°C while they are respectively -10°C and 25°C for unshorn ewes (Randall, 1993).

At arrival at the abattoir, sheep may show bruises, habitually on the back. Increased bruising risk was found to be related to more space per animal, transport on the lower deck or at the front of the vehicle and increased handling (Jarvis and Cockram, 1994). Unloading is generally easy if the ramp is stable and has an easy-grip surface and no sharp angles or corners. Lairage at the abattoir represents a novel environment, but this novelty does not appear to have adverse effects on behaviour, blood chemistry or intake of water or familiar feed in indoor sheep as no differences were found with transported sheep introduced in a familiar environment (Burritt and Provenza, 1997; Cockram *et al.*, 2000). There are some indications, however, that outdoor sheep may be more reactive to novelty or transport (Cockram *et al.*, 2000).

For hygiene reasons, sheep are often washed at the abattoir. According to Petersen (1978) this is one of most stressful procedures of the pre-slaughter period. In the spray-washing system sheep are washed for 10–15 min with a hosepipe directed on to their bellies or with an overhead shower. In the dunk-washing system, an elevator lowers the sheep in a bath for 1 min. Swim-washing is particularly stressful. Sheep are forced to swim the length of a 15 m bath and may drown when they go the wrong way and cross the following batch (Gregory, 1998). It induces bruises on the carcass and it is associated with high ultimate pH, indicative of stress or physical effort (Petersen, 1978; Bray *et al.*, 1989). Parrott *et al.* (1994) showed an increase of plasma cortisol concentration in sheep placed in a pen containing 25 cm of water as early as the 10 first minutes of the test.

The penetrating captive bolt and electrical stunning are the most common stunning methods used for sheep. Hornless sheep should be shot at the highest point of the head, to induce an immediate and irreversible loss of brain activity (Daly *et al.*, 1986; Daly and Whittington, 1989). For horned sheep, due to the thickness of their skull, the poll position should be used. Shooting in the poll position alters the mechanics of the impact and is less efficient. It can be followed by recovery of brain function and a return to sense after 33 s (Daly and Whittington, 1986). Therefore, poll-shot animals in particular should be stuck very rapidly, severing both common carotids and external jugulars, which induces loss of brain responsiveness after 14 s on average (Gregory and Wotton, 1984b). The advantage of electrical stunning is that it can be carried out on unrestrained animals kept within their group. The difficulty is to place the electrodes correctly, behind the eyes at the base of the ears, because in this context sheep keep their heads low while staying close together (Velarde *et al.*, 2000; Anon, 2004). In addition, sheep accidentally coming in contact with an animal that is being stunned, or having incorrectly positioned electrodes, can receive painful shocks (Gregory and Wotton, 1984a). As for poll-shot sheep, this type of stunning is reversible and sheep should be stuck very rapidly. It is often necessary to use pointed, wet electrodes to penetrate the wool and to establish good contact with the skin (Anon, 2004).

Farmed fish

Until recently, it was believed that fish are unable to feel pain or to suffer, because they lack the cerebral cortical structures that are involved in human pain perception and awareness. More recent studies, based on neuro-anatomical evidence and behavioural expression suggest that like mammals, fish experience pain, fear and stress (Mok and Munro, 1998; Portavella *et al.*, 2002; Sneddon, 2003; Chandroo *et al.*, 2004). Consequently, studies addressing fish welfare at slaughter have started much later compared to land animals (Figure 1).

Fish are slaughtered either near the rearing cage or tank, or transported in a transport tank over a short distance to a private abattoir on the site, or over variable distances to a specialised fish abattoir. Depending on the temperature, a period of fasting of one to several days is necessary to empty the contents of the digestive tract and to maintain a good quality of water during transport. At present, there are no European standards for stocking density at the time of transport; the acceptable maximum density depends on the species, on the transport duration and on the quality of water. The latter depends on the presence or absence of water re-oxygenation equipment on the lorry (Wedemeyer, 1992). For fish, various stunning and slaughter methods may be chosen, depending on context and species, for practical and physiological reasons and consumer requirements.

The gathering and loading procedures preceding slaughter are associated with netting, handling, crowding, changes of environment and sometimes with a temporary extraction from the water or changes in water quality and are stressful for fish (Miles *et al.*, 1974; Specker and Schreck, 1980; Cnaani *et al.*, 2004). Handling and confinement induced increased secretion of adrenaline and corticosteroids in the salmon, while struggling and hypoxia resulted in increases in the plasma corticosteroids. Metabolic disturbances, involving changes in plasma levels of glucose and free fatty acids and often decreases in hepatic glycogen levels, occurred as secondary effects of the hormonal changes (Mazeaud *et al.*, 1977; Hemre and Krogdahl, 1996). During the pre-slaughter period, as with mammals and birds, potential stress factors occur often simultaneously. It was found that in fish, their physiological effects may interfere. For example, crowding was found to be stressful, but the associated deteriorated water quality blunted the acute hormonal stress responses. Compensatory increases in the plasma cortisol were observed during the recovery period of the salmon (Pickering and Pottinger, 1987), but not in the trout (Danley *et al.*, 2005). Stress levels due to gathering and loading depend on many factors. In tilapia and trout, stress reactivity to exposure to air or handling differs between species, and also between sexes and reproductive status/season (Pottinger and Carrick, 2000; Cnaani *et al.*, 2004). In addition, different loading techniques are used for different species (e.g. pump, scoop or carpet) and do not induce the same level of stress (Wagner and Driscoll, 1994; Helfrich *et al.*, 2004).

During transport, as at other times, water quality is crucial for fish welfare. Dissolved oxygen is essential for fish respiration. The critical level is defined as the threshold value above which oxygen consumption is independent of concentration. Below the critical level, oxygen consumption is increasingly restricted by concentration, until it reaches the lethal level. Critical and lethal levels depend on fish species. They are believed to be higher in cold-water fish due to a differently shaped oxyhaemoglobin dissociation curve (Boyd, 1982). In addition to CO_2, fish excrete nitrogen as ammonia, which reacts with water to form ammonium ions in an equilibrium reaction. Un-ionised ammonia is toxic for fish. Both low dissolved oxygen or high un-ionised ammonia levels may provoke physiological stress responses and escape reactions (Pickering *et al.*, 1991; Danley *et al.*, 2005). When carried out under good water quality conditions, transport can allow recovery in certain species, resulting for example in a decrease in the plasma cortisol levels (Maule *et al.*, 1988). Oxygen levels and temperature should be similar to those experienced during farming. An adequate period of food withdrawal helps to maintain levels of carbon dioxide and nitrogen low. After unloading, the recovery time, indicated, for example, by resumption of eating activity, depends on the species (Bandeen and Leatherland, 1997).

Fish are stunned before slaughter, or directly killed. Table 2 gives an overview of the most commonly used stunning and killing methods for different fish species. Exsanguination by gill cutting without stunning induces death by ischaemia. It is routinely used in some regions to kill certain species (salmon, large rainbow trout, cod, turbot and channel catfish; Anon, 2004), but is not considered

Table 2 *Comparison of methods used for fish slaughter*

Fish species, size	Slaughter methods tested and relative levels of stress response	Stress criteria measured	Reference
Atlantic salmon (65 cm)	Electricity $\approx$ Percussion $<$ CO_2	Onset of rigor	Roth *et al.* (2002)
Eel (150–200 g)	Electricity + Oxygen removed from water $<$ Salt followed by evisceration	Early post-mortem pH	Morzel and Van de Vis (2003)
Rainbow trout (190–270 g)	Percussion $<$ Electricity $<$ CO_2	Early post-mortem pH	Azam *et al.* (1989)
Rainbow trout (300 g)	Percussion $<$ Ice slurry	Freshness (sensory evaluation, *K*-value)	Ozogul and Ozogul (2004)
Sea bream (320 g)	Percussion + immersion in ice slurry $<$ Immersion in an ice salt-water slurry $<$ asphyxia in air	Early post-mortem pH	Tejada and Huidobro (2002)
Turbot (500 g)	Percussion $<$ Bleeding in ice slurry $<$ Electricity	Early post-mortem pH Onset of rigor	Morzel *et al.* (2003)
Turbot (1 kg)	Percussion $<$ Ice slurry no bleeding $<$ Bleeding in ice slurry	Early post-mortem pH Onset of rigor	Ruff *et al.* (2002)

acceptable from a welfare point of view as it induces vigorous reactions and brain responsiveness was lost only after 148 s or more in Atlantic salmon (Robb *et al.*, 2000). Asphyxiation by removal of fish from the water is used to kill small farmed fish such as trout and tilapia. Time to die depends on ambient temperature and on species and varies between several minutes to hours (eels). It induces vigorous reactions and is considered bad for fish welfare (Robb and Kestin, 2002). Percussive stunning can be applied manually with a plastic club or 'priest', or by a semi-automatic system. The manual application requires precision and force, which can decrease after approximately 30 min of practice. Semi-automatic systems require manual introduction of the fish. They are equipped with a hammer, which is triggered by the snout of the fish. Some fully automatic systems also exist, where fish are introduced mechanically. When correctly used, these percussive stunning devices can induce immediate, permanent loss of brain responsiveness, and the stunning method is considered humane (Kestin *et al.*, 1995; Marx *et al.*, 1997; Robb *et al.*, 2000; Roth *et al.*, 2007). Compared to other commercial killing methods, percussive killing is associated with lower physical activity, as indicated by slower post-mortem muscle acidification and a slower onset of rigor mortis (Azam *et al.*, 1989; Marx *et al.*, 1997; Morzel *et al.*, 2003). Due to variations in anatomy or skull morphology, percussive stunning cannot be used for all fish species but is useful for salmonids and halibut (Van de Vis *et al.*, 2003).

Spiking is a killing method based on the insertion of a spike into the brain either manually or using specially developed equipment, causing irreversible brain damage. It uses equipment similar to the captive bolt in land animals and induces immediate loss of movement and consciousness in sea bream, salmons and eel (Nakayama *et al.*, 1996; Robb *et al.*, 2000; Van de Vis *et al.*, 2003). It is more difficult to apply than percussive stunning as it requires a technical control of the gesture. The use of anatomical markers, such as the pineal window in tuna, may be important to target the brain with accuracy (Anon, 2004). The method is reserved for species (salmon, tuna) of sufficient size (4–5 kg), which can be maintained individually (Robb *et al.*, 2000).

The immersion in water saturated with carbon dioxide is used for salmon and trout and has negative consequences for fish welfare (Van de Vis *et al.*, 2003). It causes strong behavioural responses, changes in glucose and plasma cortisol levels and in the heterophil/lymphocyte ratio and induces death only after 5 min (Marx *et al.*, 1999; Robb *et al.*, 2000). Electrical stunning consists of the application of a current through the water and is used for various species. If the chosen electrical parameters are adapted to the species, loss of consciousness may be induced in trout, salmonids and turbot (Morzel *et al.*, 2003; Roth *et al.*, 2003; Van de Vis *et al.*, 2003; Lines and Kestin, 2004). Certain species, such as rainbow trout, gilthead sea bream, sea bass and African catfish, are also commonly asphyxiated in ice or in ice/water slurry (Huidobro *et al.*, 2001; Robb and Kestin, 2002). Live fish are packed in flake ice or added to the ice/water slurry, following which water is drained off, usually after 10 min. The cold environment leads eventually to the loss of brain function, but generally acts slowly (Robb and Kestin, 2002). In addition, there is growing evidence that rapid temperature changes or the introduction to iced water is stressful for fish. Plasma cortisol and heart rate increase and fish may show escape behaviour (Donaldson, 1981; Skjervold *et al.*, 2001; Van de Vis *et al.*, 2003).

Some fish species have specific morphological or physiological characteristics, making it difficult to stun and kill them. Eels are an example but recent work found that electrical stunning can give good results (Robb *et al.*, 2002). In some countries, it has been common practice to pour dry salt (NaCl) or a 1% ammonia solution over them. The eels make extremely vigorous escape attempts. The main purpose is to de-slime them. However, if left, the chemicals may render the eels also immobile and suitable for processing, inducing unconsciousness and death, albeit after more than 10 min (Kuhlmann and Munkner, 1996; Lambooij *et al.*, 2002; Van de Vis *et al.*, 2003). The use of salt baths in conscious eels is criticised for animal welfare reasons, and prohibited in Germany since 1999 (TierSchlV, 1999). Another, smaller scale technique to kill eels is decapitation. However, studies found that severed eel heads lose brain reactions only after 13 to 30 min (Verheijen and Flight, 1997; Van de Vis *et al.*, 2003).

The best-adapted stunning method depends primarily on the species. For example, according to their habitat, some fish are very resistant to cold (immersion in icy water) or to low oxygen concentrations. Perhaps most promising for fish welfare at slaughter is the possibility to combine different stunning techniques. Live chilling has been used to immobilise fish before killing them with other techniques. In this case, salmon were kept in oxygenated, chilled sea water and then carbon dioxide stunned (Erikson *et al.*, 2006; Roth *et al.*, 2006). Similarly, eels could be rendered unconsciousness immediately and until death by applying electricity in combination with nitrogen gas to the water (Van de Vis *et al.*, 2003). This shows that to minimise stress, fish stunning and slaughter techniques urgently need further studies, for existing as well as for newly farmed or consumed fish species or sizes.

Finally, similar to other species, fish were found to be coherent in their responsiveness to stress and their levels of aggressiveness (Overli *et al.*, 1999; Tort *et al.*, 2001; Brelin *et al.*, 2005). Cluster analysis found that 6 pro-active and 17 reactive individuals could be selected from an experimental stock of 99 brown trout (Brelin *et al.*, 2005). This suggests that, similar to fowl, pigs and cattle (see above), inherent differences in responsiveness to transport and stunning/killing techniques exist in fish with a similar genetic and rearing background.

Concluding remarks and possible aims for future research

Although slaughter conditions have much improved during the last tens of years, further progress is still needed.

For fowl, food deprivation, bird catching and crating, duration and climatic conditions of transport and of lairage at the abattoir and shackling of the birds before electrical stunning are the main factors affecting stress at slaughter. Possible stress due to inhalation of stunning gas deserves more study. For pigs, the main causes of stress at slaughter are related to fighting during mixing of pens, loading and unloading conditions and the quality of the stunning procedure. Food deprivation, handling and novelty of the situation contribute to the stress reactions. For veal calves and adult cattle, disruption of the social group, handling, loading and sometimes unloading conditions, fatigue, novelty of the situation and, specifically for calves, mixing with unfamiliar animals are known stress factors. For lambs and sheep, gathering and yarding are stressful if they are extensively reared and when shepherd dogs are used. Subsequent transport may induce fatigue especially if they are commercialised through auctions or markets. Washing is also stressful for sheep. For farmed fish, known stress factors are predominantly related to environmental aspects such as temperature, oxygen, cleanliness of the water and, to a certain extent, stocking density and removal of the fish from the water. Often, stunning and killing techniques protect fish welfare insufficiently. If transport and lairage conditions are good and their durations not too long, they may allow pigs, fish, sheep, calves and adult cattle to rest. For certain species, it was shown that individuals show inherent differences in responsiveness to the slaughter procedures and that genetic origin and earlier experience influence reactions to the slaughter procedure.

Good knowledge of stress reactions at slaughter is important not just for ethical reasons. Behavioural and physiological reactions to slaughter conditions influence ante- and post-mortem muscle metabolism and consequently technological and/or sensory meat quality of fowl. Thus, chickens that were slaughtered using a more stressful procedure had altered ultimate pH of thigh meat and chickens that performed more wing flapping in response to shackling had a faster pH decline, compared to their counterparts (Debut *et al*., 2003; Berri *et al*., 2005). Similarly, Large White pigs that fought more or were more stressed during the slaughter period had higher ultimate pH than their less-stressed counterparts (Terlouw *et al*., 2005; Terlouw and Rybarczyk, 2008). Stress at slaughter resulted in higher ultimate pH in calves and adult cattle (Kenny *et al*., 1987; Lensink *et al*., 2001; Mounier *et al*., 2006), and in lambs, it increased the rate of pH decline and ultimate pH, as well as the ability of muscle to hold water (Simmons *et al*., 1997; Bray *et al*., 1989; Bond *et al*., 2004; Bond and Warner, 2007). Slaughter stress affected further flesh colour and texture in different fish species (Sigholt *et al*., 1997; Jittinandana *et al*., 2003; Morzel *et al*., 2003). Slaughter increases muscle activity as well as psychological stress and, at present, little is known of how behavioural and physiological responses interact to modify post-mortem muscle metabolism. Effects are partly related to changes in protein degradation. In fish, soft texture was associated with accelerated degradation of collagen and cyto-skeletal proteins (Sato *et al*., 2002; Morzel *et al*., 2006). In lambs, pre-slaughter exercise-induced increases in drip loss were related to increased post-mortem proteolytic activity (Bond and Warner, 2007). Finally, shocks, hits and fights have negative effects on carcass quality (bruises, lesions, bone fractures, haematomas).

Today, further progress with respect to animal welfare during slaughter procedures needs to be achieved at different levels. At the field level, efforts to install adequate loading, unloading and stocking equipment on the farm and at the slaughterhouse should continue. In the abattoirs, adequate, that is context- and species-specific designed corridors and races will facilitate movement of the animals and consequently reduce stress of the animals and work strain of the abattoir personnel. Climatic conditions during transport and lairage should be better controlled, especially for fowl and fish. For all species, procedures should be optimised such that transport and lairage durations are not too long and mixing is avoided. Abattoirs should employ skilled and trained personnel as much as possible and carefully maintain their stunning equipment.

Scientific research should play a crucial role in the determination of efficiency and the amelioration of stunning methods, of tools to assess animal welfare under field conditions and in the development of new stunning methods, for all species but especially for fish, including newly consumed fish species and sizes. Over the past decennia, a lot of effort has been put into the description of adaptive responses to slaughter procedures of animals, sometimes of different genetic or rearing background. This work allowed identifying the main sources of stress at slaughter and its consequences on behaviour, physiology and main indicators of meat quality. Such approaches use, however, limited numbers of animals. Today, new methodologies allow scientists to study these problems from a larger and more fundamental perspective. The meta-analysis currently taking place in the European Q-porkchains project (reference number 36245) establishes, among others, relationships between animal and production aspects and responses to different slaughter contexts, determining their impact and robustness, using a large data set derived from existing published results. Metabolomic, transcriptomic and proteomic studies screen RNA, proteins and other molecules present in the cell and may be used to identify the effects of stress at slaughter at the cellular level and predict effects on meat quality (cf. Morzel *et al*., 2008).

Studies that compare reactivity profiles of animals determined during tests with their reactions to the slaughter procedure help to identify the basic aspects of slaughter procedures that cause stress responses, such as human presence or novelty (Fordyce *et al*., 1988; Lensink *et al*., 2001; Terlouw and Rybarczyk, 2008). These studies allow to further predict which individuals are likely to be more reactive to slaughter procedures and pave the way to study the role of genetic background and history in the construction of the reactivity profile. Finally, analytic behavioural and physiological approaches derived from human cognitive psychology and applied to the study of emotional responses in sheep have determined that in farm animals and humans, similar cognitive processes may underlie emotional experience (Désiré *et al.*, 2004; Greiveldinger *et al.*, 2007). Such studies may be used to identify the elementary criteria responsible for the stress responses at slaughter, such as the discrepancy between expectation and reality and lack of controllability or predictability.

Thus, further research should include more recently developed tools and approaches. A better understanding of animal emotions and of the establishment and role of reactivity profiles in stress responses would improve our knowledge on animal welfare matters at slaughter. Consequences of stress should be studied also at the molecular level and a larger and more fundamental viewpoint could be obtained using statistical tools, such as modelling techniques.

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