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# Chapter 1

## Quantifying animal welfare preslaughter using behavioural, physiological and carcass and meat quality measures

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### Abstract

Animal welfare and stress refer to the subjective experience of the animal, which depends on its emotional and cognitive capacities to evaluate the situation it is in, to know its body state in terms of needs, and the higher-order, conscious, processing of the information. The origin of stress-inducing factors may be psychological, such as fear, or physical, such as food deprivation. In both cases, they influence the emotional status of the animal. Due to its subjective, therefore unmeasurable, character, the assessment of animal welfare is indirect. During the preslaughter period, this assessment relies on the physiological and behavioural stress reactions and environmental context. Physiological reactions are not specific to stress; they allow any adaptive response potentially requiring increased physical activity and vigilance. Combining physiological measurements and behavioural observations in relation to the environmental context allow interpreting these different types of information in terms of stress. Physiological and behavioural reactions further influence meat quality traits, such as post-mortem temperature and pH decline, which influence other traits, such as colour and tenderness, and carcass quality characteristics, such as bruising. Individuals show a degree of consistency in their responses to stress factors. The determination of the animal's stress reactivity using standardised tests before slaughter facilitates the interpretation of the physiological and behavioural observations made in the slaughter setting. Stress reactivity determined before slaughter may predict stress reactions at slaughter, and future meat quality. Summarising, the assessment of animal welfare and stress involves the interpretation of a range of measurements on the physiology and behaviour of the animal, the environmental context, post-mortem meat and carcass quality traits, and the characteristics of the animal itself, in terms of stress reactivity and rearing and genetic background.

**Keywords:** behaviour, blood, meat quality, stress

### 1.1 Stress and welfare

#### 1.1.1 Can animals be stressed? Can they have emotions?

Stress has often been described as the state of the animal when it has difficulties to adapt to environmental or physical constraints (Broom, 1988; Fraser *et al.*, 1975). The question of adaptation is important, but animal stress is only an ethical problem if animals are capable to experience negative emotions, such as fear or frustration (Broom, 1991; Dantzer, 2002; Dawkins, 2008; Désiré *et al.*, 2002; Duncan, 1996). Can they? Studies on behaviour, physiology as well as on the anatomy and functioning of the brain indicate that this is likely (Boissy *et al.*, 2007a; Panksepp, 2005; Paul *et al.*, 2005). First, there are many neurological similarities between humans and non-human

mammals. Particularly, the human brain contains a network of structures, called the limbic system, which processes emotions. Non-human mammalian brains also contain a limbic system, consisting of the same structures as in humans and involved in many behavioural reactions during stressful situations (Damasio, 1998; LeDoux, 2000; Panksepp, 2005; Box 1.1).

### **Box 1.1. Emotions and the limbic system.**

Small, specific lesions in the limbic system modify the expression of emotions, sometimes very specifically. For example, the amygdala, a small nucleus in the lower parts of the forebrain and part of the limbic system, is involved in fear. Blocking its functioning stopped the immobility response of rats to fear-inducing stimuli, such as cat odour (Chen *et al.*, 2006; Li *et al.*, 2004). In Rhesus monkeys, blocking the amygdala reduced fear responses to snakes (Elorette *et al.*, 2020). Humans with amygdala damage do not exhibit physiological defence responses to threat (LeDoux, 2017). Despite the specific functions of specific structures of the limbic system, the processing of emotions is probably best described as the output of the integrative functioning of the limbic and other networks, including the cortex. For example, the experience of fear involves the processing of fear stimuli not only by the amygdala, but also by structures involved in memory and arousal, amongst others (LeDoux, 2017; LeDoux and Brown, 2017).

Second, humans and animals show similar behavioural and physiological responses to stress-inducing situations, like avoidance, immobilisation or aggressive responses to danger, and startle responses to sudden events, associated with increased heart rates, amongst others. It is further thought that animals may also experience positive emotions (Boissy *et al.*, 2007b; Finlayson *et al.*, 2016; Panksepp, 2005; Veissier *et al.*, 2009). For instance, young lambs receiving regular tactile contacts from humans showed behavioural and physiological signs of a calmed state while they were stroked (Coulon *et al.*, 2015).

Both negative and positive emotions help the animal to optimise its chances to survive and reproduce, that is, to adapt, because emotions are important drivers of motivation. Animals tend to avoid situations that result in negative emotions and are attracted to those that provide positive emotions. For example, animals avoid fear or pain-inducing situations and are attracted to pleasurable experiences, such as eating food when they are hungry or shelter when they need rest. The environment and the physical needs of the animal change constantly, consequently, its emotional state, that is, its collective negative and positive emotions, also.

The capacity of animals to experience emotions is best illustrated in a series of experiments inspired by studies on emotions in humans. In human research, analytical approaches indicate that the emotion elicited by a situation depends on several well-defined characteristics of the situation, such as suddenness, familiarity, predictability, pleasantness and conformity with expectations (Scherer, 2001). For example, in humans, a situation with a high degree of suddenness, and low familiarity, predictability, controllability and pleasantness evokes negative emotions, such as fear and anger (Scherer, 2001). It was shown that animals are also capable of recognising these characteristics (Veissier *et al.*, 2009). For instance, when a piece of textile fell rapidly and unexpectedly behind the trough from which sheep were eating, they showed startle responses, asymmetric ear postures and

a sharp, short-lasting rise in heart rate, all indicative of a change in emotional state. When the object fell slowly, they did not show these reactions (Désiré *et al.*, 2004, 2006). When the appearance of the textile was predicted by a light signal, the sheep reacted less strongly (Greiveldinger *et al.*, 2007). Similarly, they looked more often at an object with their ears oriented forward when the object was unfamiliar rather than familiar (Désiré *et al.*, 2006). These results show that sheep are sensitive to suddenness, predictability and familiarity. Sheep can also form expectations. Sheep were trained to interrupt a light beam with their muzzle to receive a large food reward. When the amount was changed into a small reward, they adopted an asymmetric ear posture at the time of the reward delivery, increased locomotion and showed an increase in heart rate, indicative of an emotional response. Sheep responses to an increase in the reward were less pronounced (Greiveldinger *et al.*, 2011). It is likely that most or possibly all mammals are sensitive to the suddenness, unfamiliarity and predictability of their environment and, hence, are capable of a range of emotions, such as fear, anger, rage, despair, boredom, disgust, and happiness (Veissier *et al.*, 2009).

The above indicates that in the context of stress, while certain environmental and physical events do need adaptive responses from the animal, they are likely to provoke also negative emotions. It is important to realise that the state of stress of the animal depends only indirectly on the environment. It depends directly on the evaluation of the environment by the animal. The animal is stressed, that is, feels negative emotions, when it feels threatened, whether the threat is real or not. In order to adapt to this threat, the animal reacts behaviourally, with flight or defence reactions for example, associated with physiological changes, such as a faster heart rate and hormonal changes, which allow the increase in physical effort and vigilance (Terlouw, 2005; Veissier and Boissy, 2007).

### 1.1.2 Animal welfare and mental states, even more complex!

The concept of animal welfare is larger than of the concept of stress. While emotions are fluctuating and of short duration, the welfare depends on the global emotional state of the animal, as well as on its desires and preferences, considered over a longer time period (Boissy *et al.*, 2007b; Dawkins, 2003). Events with strong negative emotional impacts may have long-lasting effects on the global emotional state, and physiological and behavioural functioning, not only in humans, but also in animals. This was demonstrated in lambs that during rearing were subjected to repeated, unpredictable and uncontrollable aversive events, such as social separation, changes in the environment, noise, and handling. These lambs were subsequently trained in a choice test where they received a reward if they chose the correct bucket (e.g. the bucket placed on the left) and were punished if they chose the incorrect bucket (the one on the right). The final test consisted of placing the bucket in the middle, which is an ambiguous position. Lambs that had been subjected to repeated negative events during rearing chose the ambiguous position, 'middle', less often than their unstressed counterparts. This indicates that the way these lambs evaluated the situation had a negative or pessimistic bias compared to the unstressed controls (Destrez *et al.*, 2013).

Certain authors consider that the expression of self-awareness, memory, symbolic language, social understanding, and anticipating the behaviour of, or attributing desires to, conspecifics ('mindreading'), as well as having interests, matter for animal welfare (Jones, 2013; Krupenye and Call, 2019). The above considerations imply that at least some non-human mammals have complex,

subjective, mental states. Today, many scientists believe that, like humans, non-human mammals construct an integrated mental image from the information perceived from the environment, the body and from the knowledge, they have through memories and other sources (Damasio, 2010). The construction of such an image needs a range of processes involved in the acquisition, storage and manipulation of information, referred to as cognition (Mendl and Paul, 2004; Box 1.2). The constructed mental image is foundational for the rich emotional, physiological and behavioural reactions of mammals to varying needs and a changing environment.

### Box 1.2. Cognitive abilities of animals.

Emotional contagion refers to an emotional state in an observer as a direct result of perceiving the emotional state of another. Empathy refers to situations in which the observer has a similar emotional state as another being as a result of the perception of this being's situation. The processing underlying empathy is more complex than for emotional contagion. In contrast to emotional contagion, the distinction between self and other is maintained, and the emotional state remains focused on the other being rather than self-focused (Preston and De Waal, 2002). Examples of empathy are described for apes, elephants and dolphins (De Waal *et al.*, 2006). Emotional contagion has been described in other species (De Waal, 2008). For example, pigs, when joining another, stressed pig, showed behaviour that was different from when joining a non-stressed pig (Reimert *et al.*, 2015).

At least certain primates are capable of metacognition, that is, they are capable of judging their own state of knowledge. For example, Rhesus monkeys were trained to choose among four opaque tubes, one of which concealed food. On only half the trials, the monkeys observed the experimenter baiting the tube, so that they knew which tube contained the reward. They were allowed, however, to look down the length of each tube, before choosing. When they had seen the experimenter baiting, most monkeys chose without looking; when not, most often they looked in the tube first. This suggests that they discriminated between knowing and not knowing (Hampton *et al.*, 2004). Another experiment allowed dolphins performing a discrimination task, to choose the 'opt-out option'. Choosing this option, the dolphin received a greater reward than the reward given for an incorrect response, but lower than the reward given for a correct response. The dolphins seemed to choose this option when they had a bigger probability of failing the task (Smith *et al.*, 1995). Similar results were found in rats and pigeons (Adams and Santi, 2011; Foote and Crystal, 2007). However, it is believed that metacognition requires self-reflective consciousness and certain scientists question the existence of such capacities in non-primates, because more simple behavioural principles may also explain the observed behaviour, such as information seeking behaviour (Foote and Crystal, 2012; Kirk *et al.*, 2014; Smith *et al.*, 2008). Particularly, rats and bees accept to work for information on where food can be found, and to work harder if the food is more difficult to find (Foote and Crystal, 2012; Kirk *et al.*, 2014; Lehrer, 1993).

Helping behaviour is a social behaviour whereby an individual helps another irrespective of disadvantages to itself. Again, there are many examples in primates (Preston and De Waal, 2002). There is also recent evidence for helping behaviour in rats. Rats quickly learned to free a rat trapped in a wet area, and did only so if the wet rat showed signs of distress. When rats were forced to choose between opening the door to help a distressed cage mate and opening a different door to obtain a food reward, in most test trials, rats chose to help the cage mate before obtaining a food reward (Sato *et al.*, 2015).

Theory of mind is the ability to ascribe mental states, such as desires and beliefs, to others. While it is central to human social life, current research indicates that other mammals have also a theory of mind (Krupenye and Call, 2019; Van der Vaart and Hemelrijk, 2014). Most work was carried out on apes and monkeys, but dogs and corvidae have similar capacities. For example, Western scrub jays show caching behaviour: they bury food items for future consumption.

Conspecifics will steal such items if they see them being hidden, but cachers take measures to prevent such theft. When given worms to cache, with a visible competitor in an adjacent cage, they re-cached their worms in new sites once they were alone, but only if they had previous experience being pilferers themselves (Emery and Clayton, 2001).

As for emotions, the arguments for the existence of complex mental states in non-human mammals are based on analogies between humans and other mammals. In humans, the construction of complex mental states needs widespread functional connectivity between different brain areas, including the prefrontal cortex, necessary for higher order information processing (Salzman and Fusi, 2010). The question is whether non-human mammals have brain structures and connections allowing such complex processing. There are many similarities between humans and other mammals. Monkey and human brains contain essentially the same prefrontal cortex areas, but certain prefrontal cortex areas do not exist in rats for instance (Öngür and Price, 2000; Rolls, 2015). Despite these variations, in non-human mammals, areas of the prefrontal cortex make widespread connections with other cortical areas, and information processing subcortical areas, including the limbic system (Öngür and Price, 2000; Rolls, 2015). This suggests that all mammals have the necessary connections between the relevant brain areas to have at least some degree of higher-order information processing, allowing the construction by the brain of complex mental states that include emotions.

### 1.1.3 Can animals suffer? Are they conscious?

The above information indicates that non-human mammals may be capable to experience negative emotions and to construct complex mental states by higher-order processing of information. Scientists have argued, however, that these capacities are necessary, but not sufficient conditions for animal welfare matters to be an ethical problem (Mendl and Paul, 2004). Ethical questions related to animal welfare infer that animals are able to suffer and to experience pleasure. These scientists argue that the central question is whether animals have conscious knowledge of their sensations and emotions. The question has been debated for many years (Birch *et al.*, 2020; Dawkins, 2015; Mendl and Paul, 2004). Initially, the question was whether animals are conscious. Over the last ten years, the scientific understanding of human consciousness and the vision of animal consciousness have much evolved. Conceptual progression, clever experimental paradigms and new, sophisticated brain imagery techniques were major drivers (Lamme and Roelfsema, 2000; Laureys *et al.*, 2004; Tononi, 2012). In 2012, the Cambridge Declaration on Consciousness declared that humans are not the only conscious beings and that 'non-human animals, including all mammals and birds, and many other creatures, including octopuses' possess neurological substrates complex enough to support conscious experiences (Birch *et al.*, 2020). Today's question is not *if*, but *how* animals are conscious. A recent paper considers the possible existence of different, multidimensional consciousness profiles, varying across species, including birds and fish (Birch *et al.*, 2020; Box 1.3). It can be argued on the same grounds that each *individual* has its own consciousness profile.

### Box 1.3. Higher-order processing in birds and fish.

Like mammals, birds express complex cognitive behaviour. They can classify images according to their content or group them according to abstract concepts, such as 'similar' or 'different'. Pigeons, for example, distinguish photos containing humans from those that do not (Emery, 2006). Pigeons, hens and quails are also able to solve complex problems that require the application of a rule (Emery, 2006). The best performances are observed in corvids and parrots (Gossette *et al.*, 1966). Birds do not have a cortex, like mammals do, but their brain contains a structure called pallium, showing similarities in connectivity and functional organisation to the mammalian cortical structures (Jarvis *et al.*, 2005). The avian pallium receives information relative to the environment and the body state and is capable of higher order processing of information, explaining their cognitive abilities (Jarvis *et al.*, 2005; Nomura and Izawa, 2017).

Fish lack the cerebral cortical structures that are involved in consciousness in humans. Despite this, neuro-anatomical evidence and physiological and behavioural reactions suggest that fish experience pain, and other forms of negative emotions, including fear (Chandroo *et al.*, 2004; Mok and Munro, 1998). Behavioural observations indicate that teleost fish have a number of learning and memory mechanisms and basic cognitive capabilities, similar to those in mammals, birds and reptiles (Broglio *et al.*, 2005). For instance, trout that receive a painful stimulus change their behaviour by rubbing the painful site against available substrates (Sneddon, 2003, 2009). In goldfish, lesions in a specific area of the brain (the dorsomedial telencephalon) affected emotional pain-related behaviour, as the fish no longer avoided a painful stimulus (Portavella *et al.*, 2002). These scientists conclude that, although the fish brain lacks certain structures of the mammalian brain involved in higher-order processing of information, other structures may fulfil these functions. Other scientists claim however, that such results show that fish respond to noxious stimuli but do not necessarily experience pain (Rose *et al.*, 2014).

Despite these recent evolutions, sceptics maintain that unequivocal evidence of existence of higher-order mental states in animals is still wanting (Krupenye and Call, 2019; Box 1.2). Other scientists argue that materialistic approaches of science cannot elucidate the existence of consciousness (Beauregard *et al.*, 2014). They indicate that quantum mechanics have shown that atoms and subatomic particles are not really solid objects as they are essentially empty. Furthermore, they do not exist with certainty at definite spatial location at definite times. These scientists indicate that our vision of reality is exclusively materialistic and therefore incomplete. They argue that the mind is not derived from matter. Most scientists acknowledge the difficulty to provide formal proof for higher-order mental states in animals, as it is impossible to get access to the subjective state of another being, but they argue that, for the sake of precaution, we should assume that animals consciously perceive their inner mental state, including emotions (Mameli and Bortolotti, 2006; Mendl and Paul, 2004; Box 1.4).

### Box 1.4. Are you sure that your neighbour has consciousness?

The question of the existence of higher-order states can be asked both for animals and humans. Most persons consider having emotional and complex mental states and believe that other persons have similar states. However, as these states are exclusively personal and subjective experiences, how to know that this is true? Can we prove that other humans are conscious? Would the world function in the same way if humans did not have any subjective

experience, or no consciousness? This is a subject of much philosophical debate, opposing materialistic and non-materialist points of views, amongst others (Chalmers, 1996; Dennett, 1991; Hyslop and Jackson, 1975; Wittgenstein, 1958).

In conclusion, animal welfare and animal stress are subjective states, that is, they depend on the animal's conscious experience of its inner state. This inner state can only be measured indirectly, essentially by evaluating the behavioural and physiological expressions of these states, or the longer-term consequences of these expressions.

## 1.2 Studying and measuring stress

To measure and understand stress responses, it is useful to distinguish the potential causes of stress according to their origin: psychological or physical. Those of a psychological origin, such as fear, frustration, resignation and sadness, result from thwarted desires and preferences (Boissy, 1995; Destrez *et al.*, 2013). During the preslaughter period, stress of psychological origin is often caused by social disturbances, sudden events and the unfamiliarity of the different situations the animals encounter. They are due to the departure from the farm, the introduction into the unfamiliar environments of the lorry and the abattoir, the exposure to sounds and humans, and the disruption of the social group, due to separation and mixing of unfamiliar animals (Terlouw *et al.*, 2008).

Those of a physical origin inflict physical constraints on the functioning of the body, such as a state of hunger and thirst, fatigue, illness or tissue lesions. In the preslaughter context, they may result from food and water deprivation, negative events during transport and lairage, including physical compression, extreme temperatures, collisions with pen fittings or other animals, various interventions by humans, including the use of electric prod, and fighting between animals due to mixing, amongst others (Bourguet *et al.*, 2011b; Danziger, 2006). Despite the physical component of these stress factors, they influence also the emotional state of the animal. This was demonstrated by Bourguet *et al.* (2011a) who showed that cows and heifers that had been food deprived for 30 h, a stressor of physical origin, were more fearful to human presence and handling, and unexpected events (i.e. a light air blast on the nose), which are stressors of psychological origin. This further indicates that a given stress factor during the preslaughter period may exacerbate the effects of other stress factors on the levels of stress of the animal. Similarly, pain, which may be caused by fighting or bruising, for example, has a strong negative affective component; it is this negative experience which makes that painful situations are avoided (Villemure *et al.*, 2003; Box 1.5).

### Box 1.5. Pain and the pain system.

Specialised neurons of the somatosensory system, called nociceptors, are activated by noxious stimuli, that is, stimuli that have the potential to cause injury. Nociceptors are found in the skin, joints, muscles and internal organs. They can respond to temperature (thermoreceptor), chemicals (chemoreceptors) and pressure (mechanoreceptors) (Millan, 1999). Other receptors are sensitive to the touch and are part of the non-nociceptive somatosensory system.

Activation of nociceptors beyond a certain threshold causes the perception of pain (Millan, 1999). The perception



of pain is associated with potential tissue damage and must be taken into account by the organism. Therefore, it is difficult to ignore pain (Eccleston and Crombez, 1999).

The nociceptors transform the harmful stimulus into a nerve message that is transmitted to other specialised neurons, called secondary nociceptors, in the dorsal horn of the spinal cord. The latter transmit their signals to various brain structures forming a network called the 'pain matrix' (Ingvar, 1999). This matrix includes structures of the brainstem, subcortical structures, and cortical areas. The pain experience results from the coordinated activity of the different regions of the brain that make up the pain matrix. Regardless of the type of pain, certain areas of the matrix are always activated during the perception of pain. Most pain matrix structures are, however, not pain specific. For example, most regions of the pain matrix also respond to tactile, auditory and visual stimuli in a similar way as to nociceptive stimuli. In addition, in humans, stimulation of structures of pain matrix, with a few exceptions, generally does not cause the experience of pain (Garcia-Larrea and Peyron, 2013; Mazzola *et al.*, 2012).

As indicated by the International Association for the Study of Pain (IASP; Raja *et al.*, 2020), the concept of pain refers to the situation where both dimensions, the sensory (the nature, intensity and duration of the stimulus) and motivational-affective (the unpleasantness) dimensions of pain are perceived. The pain sensation represents therefore a nociceptive sensation associated with a negative affective sensation. Different structures in the brain are associated with these different components.

### 1.2.1 Behavioural and physiological reactions to stress under controlled conditions

Reactions to specific stressors may be tested under controlled conditions (Table 1.1). These tests show that animals show a certain consistency in the way they react to stressors, referred to as the animal's stress reactivity (Bourguet *et al.*, 2015; Terlouw and Rybarczyk, 2008). Stress reactivity is considered high if an animal feels easily threatened and presents pronounced behavioural (whether overt or not) and/or physiological reactions. This consistency was illustrated by Boissy and Bouissou (1988, 1995; Table 1.1), who tested heifers in a series of tests confronting them with unfamiliarity, isolation, conflict between fear and motivation to feed, and social isolation. Heifers that were more reluctant to explore an unfamiliar object compared to their conspecifics were also more reluctant to feed near a fear-inducing stimulus and showed more signs of fear when they were socially isolated in an unfamiliar environment. This approach allowed the identification of several fear related behaviours, which differed according to the context (Table 1.2).

Individuals that were more fearful showed *higher* activity levels during the human exposure test, during restraint and during social isolation. However, they showed *lower* activity levels during the open field test, the novelty test and the surprise test. This shows that the behavioural fear response depends on the context. Fear induces defence and avoidance reactions, with defence reactions aiming to drive the threatening factor away from the animal, and avoidance reactions moving the animal away from the threat. The animal may also immobilise in order to remain unnoticed. Possibly, in the studies by Boissy and Bouissou (1988, 1995), the active responses expressed the motivation to escape, while the passive responses expressed the motivation to avoid approaching the threat or to remain unnoticed.

Other species show also consistent differences in stress reactivity. Gilts that accepted relatively easily to be removed from their home pen and to be separated from their social group, also accepted

**Table 1.1.** Examples of reactivity tests that examine different stress factors.

	Factors tested	Species	Reference
<b>Individual tests<sup>1,2</sup></b>			
Novel object test	unfamiliarity	sheep, pigs	Boissy and Bouissou, 1988; Désiré <i>et al.</i> , 2004, 2006; Lawrence <i>et al.</i> , 1991; Olsson <i>et al.</i> , 1999; Terlouw and Rybarczyk, 2008
Walking through a corridor	unfamiliarity	heifers	Boissy and Bouissou, 1988
Surprise test	unfamiliarity, surprise	heifers, bulls, sheep, calves	Boissy and Bouissou, 1995; Bourguet <i>et al.</i> , 2015; Désiré <i>et al.</i> , 2004, 2006; Greiveldinger <i>et al.</i> , 2009; Lensink <i>et al.</i> , 2000
Open field test	open space	heifers, sheep	Boissy and Bouissou, 1995; Deiss <i>et al.</i> , 2009
Human exposure test	human passive presence, acceptance of being approached, handled and/or stroked	bulls, sheep, pigs, calves, goats	Bourguet <i>et al.</i> , 2015; Deiss <i>et al.</i> , 2009; Lawrence <i>et al.</i> , 1991; Lensink <i>et al.</i> , 2000; Lyons <i>et al.</i> , 1988; Terlouw and Porcher, 2005; Terlouw and Rybarczyk, 2008
Restraint in a crush	lack of freedom of movement	heifers	Boissy and Bouissou, 1995
Social isolation	absence of familiar conspecifics	heifers, sheep	Boissy and Bouissou, 1995; Bourguet <i>et al.</i> , 2010; Deiss <i>et al.</i> , 2009
Resident-intruder	territorial aggression/submission	pigs, rainbow trout	Olsson <i>et al.</i> , 1999; Øverli <i>et al.</i> , 2002
<b>Group tests</b>			
Food competition test	aggression, competition	pigs	Lawrence <i>et al.</i> , 1991; Terlouw and Rybarczyk, 2008
Human: passive presence or active approach	fear due to humans	sheep	Le Neindre <i>et al.</i> , 1993
Sorting out of cattle <sup>3</sup>	human presence and interventions, restraint, social separation	bulls, cows	Fordyce <i>et al.</i> , 1988a
Mixing of unfamiliar animals	aggression, competition	pigs	Terlouw and Rybarczyk, 2008; Terlouw <i>et al.</i> , 2005, 2009
<sup>1</sup> In individual tests, if a companion animal is not visible (behind a grid or fence, for instance), the reaction to social isolation influences the response.			
<sup>2</sup> If the test is carried out in an unfamiliar environment, the reaction to unfamiliarity influences the response.			
<sup>3</sup> Often part of normal stock procedures, using a crush, races and alleys.			

**Table 1.2.** Behaviours indicative of high fear response in different tests (Boissy and Bouissou, 1988, 1995).

Test	High fear response
Novel object test	long latency to approach, long latency to exploration
Surprise test	strong startle, long latency to return to the surprise inducing object
Open field test	long latency to enter, high levels of standing immobile, high levels of head in an upright position
Human exposure test	moving away from human, low levels of immobility
Restraint	struggle, vocalisation
Social isolation	vocalisation, attempts to return to the familiar conspecifics
Walking through a corridor	refusal to walk, or in contrast, running

better to be driven through a corridor and were less reactive to the sudden approach of a human. In a novel object test, these same gilts touched and looked at the object less often than their more reactive counterparts. Furthermore, in a food competition test, these gilts won a smaller proportion of aggressive interactions, but had a greater feeding success (Lawrence *et al.*, 1991; Table 1.1). It is not clear whether the different profiles of these gilts were simply related to differences in fearfulness, or more generally in reactivity levels, irrespective of the stress factor. In sheep, certain individuals showed relatively high levels of locomotion, vigilance and bleating in several tests, including an open field test, social isolation, and exposure to human presence, compared to their less reactive group members (Deiss *et al.*, 2009; Table 1.1). Similar results were found in goats, fowl and fish (Castanheira *et al.*, 2017; Jones *et al.*, 1994; Lyons *et al.*, 1988). For instance, fish that were more reactive to confinement were also more active in the presence of an intruder and did not feed in isolation, in contrast to their less reactive counterparts (Øverli *et al.*, 2002).

Stress reactivity depends at least partly on genetic background and rearing conditions. In a surprise test comparing young bulls of different breeds, each bull was isolated in a corridor and an umbrella was presented. The umbrella was first closed, and subsequently suddenly opened. Strictly speaking, in addition to surprise, this test combines social isolation, and novelty due to the unfamiliar test environment and the unfamiliarity of the umbrella (Table 1.1). Compared to Angus, Blond d'Aquitaine bulls looked less at the closed umbrella and remained at a greater distance from the opened umbrella. Throughout the test they expressed more often startle responses and had faster heart rates. Other tests also showed breed differences. During a 'human exposure' test, the Blond d'Aquitaine bulls expressed more vigilance. These results indicate that the Blond d'Aquitaine bulls were more reactive to various stressful situations, probably due to greater fearfulness (Bourguet *et al.*, 2015). Other species also show breed differences. During a human exposure test, Duroc pigs approached and touched the human significantly more often than Large Whites. This breed difference was specific for the motivation to touch humans, because these same pigs did not differ behaviourally or physiologically during a novel object test (Terlouw and Rybarczyk, 2008; Table 1.1). Romanov sheep eliminated more, ate less and avoided a human more than Merinos sheep (Le Neindre *et al.*, 1993). Concerning rearing conditions, pigs and calves that had positive contacts with a human or the farmer during rearing, approached more easily other humans and were easier to handle, respectively (Lensink *et al.*, 2000; Terlouw *et al.*, 2005). Free-range born pigs were less aggressive during resident-intruder tests and established more quickly a clearly defined dominance/subordinate relationship. Possibly, during conflicts, the pigs reared in a confined space had not learned to withdraw and showed less avoidance (Barton-Gade, 2008; Olsson *et al.*, 1999; Terlouw *et al.*, 2009). These pigs also differed in non-social tests, such as the novel object test (Olsson *et al.*, 1999; Table 1.1).

### 1.2.2 Behavioural stress responses in the slaughter context

In abattoirs, fear is a common cause of stress and may be expressed by the refusal to go forward, walking backwards, immobilisation or standing still despite interventions by operators. Cattle may press other animals against walls or gates while walking backwards and may turn their head if the size of the single-file corridor does not allow turning of the body (Bourguet *et al.*, 2011b; Table 1.3). Fear may also cause escape attempts, resulting in running, slipping and/or falling (Table

**Table 1.3.** Examples of animal based observations in an abattoir and possible causes in terms of stress for cattle (Bourguet *et al.*, 2011b; Grandin, 2019).

Animal based observation	Cause
Retreat, attempted retreat (animals turn their heads backwards), refusal to move forward, refusal to enter certain places including the stunning area	fear of what animal perceive or expect to encounter if moving forward
Slips, falls <sup>1</sup>	fear, pain <sup>1</sup>
Vigilance (immobile, poll of the head above the shoulder, ears immobile or moving back and forward, fixed gaze directed towards a point in the environment)	perception of a potential threat or stressful event, possibly causing fear
Compressed animal (the animal has a curved back because it lacks space due to the close presence of other animals and/or space limiting objects, such as barriers or walls)	fear, pain
Tremor (rhythmic shaking of the body)	fear, pain, cold
Accelerated and/or deep breathing	fear, pain, heat stress
Vocalisation <sup>2</sup> of an isolated animal	fear of social isolation
Vocalisation <sup>2</sup> during driving or other interventions	pain due to use of the goad or due to too strong compression of the animal in the stunning box
Vocalisation <sup>2</sup> during lairage	at least in calves <sup>3</sup> : fear due to an unfamiliar environment and/or conspecifics
Kick	fear or pain if observed during driving, pain if observed during restraint in the stunning box

<sup>1</sup> Fear and pain can be the cause, but can also be the result of slipping and especially falling.

<sup>2</sup> Vocalisation of non-isolated cattle is considered a social expression, and not related to stress (Grandin, 2001).

<sup>3</sup> Similar interpretations can be made for pigs (Friel *et al.*, 2019).

1.3). Other frequent causes of stress in the preslaughter context are social separation or isolation, which may cause vocalisation, particularly in cattle and sheep (Deiss *et al.*, 2009; Grandin, 2001; Table 1.3). The responses to physical causes of stress, such as thirst, hunger and fatigue include attempts to replenish the physical needs, such as trying to get access to the water bowl, or to lie down (Jarvis *et al.*, 1996a,b; Knowles *et al.*, 1999). Generally, there is no searching for food, as food is often not available in abattoirs (Table 1.4). Known expressions of hunger are increased activity and competition, difficult handling, and vocalisation (Bourguet *et al.*, 2011a; Brown *et al.*, 1999; Dalla Costa *et al.*, 2016; Thomas *et al.*, 2001; Vieira *et al.*, 2008). In fish, low oxygen levels or high unionised ammonia levels provoked escape reactions (Danley *et al.*, 2005; Pickering *et al.*, 1991).

During the pre-slaughter and slaughter period various factors may cause pain. According to the IASP, pain is: 'An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage.' The IASP adds: 'Verbal description is only one of several behaviours to express pain; inability to communicate does not negate the possibility that a human or a non-human animal experiences pain' (Raja *et al.*, 2020; Box 1.5). Examples of potentially painful events include falling, slipping, adverse interactions between animals, such as being bitten, being head-butted, or being mounted, hitting structures, pen fittings, gates and other equipment, human interventions to drive animals forwards and unsuccessful stuns (Jarvis *et al.*, 1996b). Acute painful stimuli may cause avoidance reactions, aggression, agitation, and, at least in

pigs and cattle, vocalisation (Cordeiro *et al.*, 2018; Grandin, 2001; Marx *et al.*, 2003; Prunier *et al.*, 2013). It causes kicking in cattle (Bourguet *et al.*, 2011b; Table 1.3). In birds, painful stimulation may induce passive immobility or active avoidance behaviour, such as jumping, and occasionally, calling (Gentle, 1992). Wing flapping in birds is a sign of pain and discomfort and may be caused at shackling by poor handling and compression of the birds' hock due to tight fitting shackles, or as a result of pre-stun shocks when birds enter the water bath stunner (Gerritzen *et al.*, 2022 – Chapter 6 of this book; Gregory and Bell, 1987; Sparrey and Kettlewell, 1994; Terlouw *et al.*, 2008). Long-lasting shackling may also be very painful for the birds, causing wing flapping and sometimes broken bones (Gentle and Tilston, 2000; Gerritzen *et al.*, 2022 – Chapter 6 of this book; Gregory and Wilkins, 1990). Depending on the body site concerned, pain may also cause abnormal postures, abnormal or avoidance of walking (Prunier *et al.*, 2013). Certain reactions, such as lack of activity and prostration, are not overt and more difficult to identify (Prunier *et al.*, 2013). Bruising is an indirect indicator of pain.

Certain stress-inducing characteristics of the abattoir can also be identified by evaluating the environment itself. The environmental context may cause lack of physical comfort or fear (Table 1.4). Detailed observations involving watching the animals, while taking into consideration the context, need careful analysis to understand the causes of the behaviour, to recognise the problem, to identify the cause, and find solutions.

As indicated above, vocalisations may be simply social exchanges, or reactions to pain or hunger, depending on the situation and the species and age of the animal (Grandin, 2001; Marx *et al.*, 2003; Thomas *et al.*, 2001; Table 1.3). It is important to realise that the visual, olfactory and auditory capacities of animals are different from those of humans. For example, the eyes of farm animals adapt more slowly to changes in light conditions and light contrasts may be interpreted as obstacles (Willson *et al.*, 2021; Box 1.6).

### **Box 1.6. Vision in farm animals.**

Vision relies on a highly complex organised system. The retina is a sophisticated image processor, containing various cell types that form microcircuits that have different functions, encoding different aspects of the visual scene. The signals processed by these different retinal circuits are relayed to specific brain regions (Zhang *et al.*, 2017). Form, orientation, colour, motion, and depth are processed in discrete areas of the cerebral cortex. The number of functionally discrete areas of the visual cortex varies between species.

Mammals and birds have two types of photoreceptors in the retina, rods and cones; they contain photosensitive pigments. Rods are found predominantly in the periphery of the retina, whereas the cones are located mostly in the centre. In mammals, there are many more rods than cones in the retina. The cones allow vision of fine details (called visual acuity) and colour discrimination, but do not function under low light conditions. The rods cannot distinguish colours and have poor resolution, but they mediate vision under low light conditions. At intermediate levels of illumination, rods and cones function simultaneously (Jonas *et al.*, 1992).

As they have different characteristics, the study of amounts and distribution of rods and cones delivers important information on visual capacities. In the human retina, depending on the location, there are 30,000 to 150,000 rods/mm<sup>2</sup>, and 6,000 to 180,000 cones/mm<sup>2</sup>. As the cones are confined to a limited area, overall, humans have 20 times

**Table 1.4.** Examples of environment-based observations, possible negative consequences for animal stress and proposed solutions (Bourguet *et al.*, 2011b; Grandin, 2019).

Environment-based observations	Risks in terms of stress	Possible solutions
<b>During unloading at slaughter</b>		
Outdoor unloading facilities	Fear of novel environment and odours	Integration of the unloading area as much as possible into the lairage area, with a roof and high solid walls
Absence of equipment for injured animals after transport	Pain	Provision of pens easily and quickly accessible from the unloading area
<b>During lairage</b>		
Mixing of unfamiliar animals	Risk of aggressive behaviour, resulting in pain, fear and fatigue	Use of individual stalls or maintain only familiar conspecifics in groups
Large groups of animals	Risk of aggressive behaviour, resulting in pain, fear and fatigue	Decrease group size with more but smaller pens
Use of individual stalls with no front gate	Need to make animals to walk backwards: resulting in fear and discomfort	Installation of front gates
Absence of food <sup>1</sup>	Hunger, increased reactivity to fear-inducing factors	Improved planning to reduce lairage durations, or provision of food
Absence of functional drinkers available and accessible for each animal <sup>1</sup>	Thirst, risk of increased reactivity to stress factors	Regular checks and maintenance of drinkers, avoidance of excessive densities of the animals in pens. Adjusted height of the drinkers according to the species, the age of the animals and the type of drinkers
Lack of space	Animals unable to lie down: discomfort, fatigue, and thirst due to low accessibility of drinkers	Written indications of the maximum number of animals allowed in each pen and compliance with the indication
Inappropriate temperatures	Cold or heat stress, discomfort	Cold: install windbreakers, improve thermic isolation of the stalls or area, add litter or rubber mats Heat: use humidifiers, add openings to ventilate the stalls
Bad air quality, presence of ammonia	Discomfort	Improve the ventilation system and/or add openings to ventilate the stalls, increase the frequency and quality of cleaning of the stalls
Noise	Fear and discomfort	Limit noise pollution: Installation of rubber stoppers on the doors and sound insulation on the hydraulic systems, lubrication of cylinders
<b>During handling</b>		
Light contrasts: (1) bright light (e.g. when the truck is opened on a sunny day), (2) darkness (e.g. when the animal has to enter a dark abattoir on a sunny day) or (3) shadows on the ground (Willson <i>et al.</i> , 2021)	The animal is (1) dazzled, (2) perceives only darkness or (3) perceives shadows as obstacles which cause fear when forced to move forward	Allowance of the necessary time to the animals, so they can adapt to the light conditions if they change (indoors/outdoors). Sharp shadows should be avoided. Installation of artificial lights where needed. Use of warm colour light (above 3,000 K)

&gt;&gt;&gt;

Table 1.4. Continued.

Environment-based observations	Risks in terms of stress	Possible solutions
Irregular or insufficient light indoors	Incapacity of the animal to see ahead which will cause fear reactions	Light should be sufficient and homogeneous throughout the abattoir; for example, neon lights above and in the same direction of the animal corridors
Light reflections	Dazzling effect: fear	The operator may place her/himself at the place of the animals (eyes at the same height) to see if water or metallic objects are reflecting light (sun, artificial lights). Remove sources, paint reflecting objects, or block view
Races equipped with openwork barriers or solid walls but too low (Figure 1A, B and C).	The animal can see objects or humans outside the race, which may cause fear	Installation of solid sides or other means that prevent animals from seeing out
Individual corridors	Fear due to social isolation, risk of injury	Limitation of individual sections of corridors as much as possible in order to allow walking of the animals in shoulder-to-shoulder or flank contact
Straight corridor and/or sharp angles	Fear due to a lack of exit, the animal perceiving a dead end	Avoiding use of straight corridors and sharp angles, but rather curved corridors
Excessive downward slopes	Fear of slipping/falling	Use of gentle (<20°) slopes with non-slipping floor
Slippery floor	Risk of slipping and falling: fear, pain	Regular cleaning of the floors. Repairation of floors to make them non-slip
Floor with heterogeneous or irregular aspects (Figure 1D and E)	The animal interprets irregularities as obstacles	Repairation of floors to give them a regular aspect
Unstable or moving floor, or changes in floor level (Figure 1B)	Fear of falling	Provision of facilities with solid and stable floors
Air blast	Suddenness inducing startle and fear responses including backward movements	Move the air exhausts of the pneumatic systems out of areas where animals move (in the attic for example)
Inappropriate use of the electric goad (continuously on the same animal, on an animal that cannot move forward or that has collapsed, or on sensitive parts of the body (head, genitals, etc.) <sup>2</sup>	Pain, fear	Identification of the reason for the excessive use and correct the cause; improve equipment and conception, organisation of the abattoir, remind operators of the rules, and/or offer appropriate training
Metal doors, ungreased cylinders, uninsulated hydraulic systems	Fear and discomfort	Limit noise pollution: Installation of rubber stoppers on the doors and sound insulation on the hydraulic systems, lubrication of cylinders

<sup>1</sup> Council Regulation (EC) No 1099/2009 stipulates that operators take the necessary measures to ensure that animals:

- (a) are provided with physical comfort and protection, in particular by being kept clean in adequate thermal conditions and prevented from falling or slipping;
- (b) are protected from injury;
- (c) are handled and housed taking into consideration their normal behaviour;
- (d) do not show signs of avoidable pain or fear or exhibit abnormal behaviour;
- (e) do not suffer from prolonged withdrawal of feed or water;
- (f) are prevented from avoidable interaction with other animals that could harm their welfare.

<sup>2</sup> Council Regulation (EC) No 1099/2009 stipulates that the use of instruments, which administer electric shocks, shall be avoided as far as possible. In any case, such instruments shall only be used for adult bovine animals and adult pigs which refuse to move, and only when they have room ahead of them in which to move. The shocks shall last no longer than one second, be adequately spaced and shall only be applied to the muscles of the hindquarters. Shocks shall not be used repeatedly if the animal fails to respond.



**Figure 1.1.** (A) Entrance of a pig restrainer. The negative points are that the area is too dark; the environment outside the restrainer is visible causing too many distractions for the animal; the false-floor is lacking in the restrainer, while it is known that the absence of a floor causes fear; and metal floors are inappropriate because they may vibrate and be noisy. (B) Cattle identification crush. The negative points are that the environment may be difficult to interpret for the animal because there are too many objects and shapes; the walls are too low allowing vision of the outside environment distracting the animals; there is insufficient light, with dark areas; the floor is non-homogeneous and there is a step at the entrance of the crush which may present obstacles for the animal; and the one-way backstop is little efficient. (C) Sheep entrance of a single file race towards the slaughter area. The negative points are that the reduction in the size of the entrance of the race is too abrupt; the environment outside of the race is too light, possibly distracting the animal; and the aspect of the floor changes which the animal may perceive as an obstacle. (D) Entrance of a pig restraining box. The negative points are that the corner is too sharp; the aspect of the floor changes which the animal may perceive as an obstacle; metal floors are inappropriate because they may vibrate and be noisy; and the concrete floor does not have a non-slip surface. (E) Sheep entrance to the slaughter area. The negative points are the complexity of the environment, which may be difficult to interpret for the animal; the presence of the water hose and the changing aspect of the floor, which may present obstacles for the animal. (Photo courtesy of C. Bourguet, Bureau ETRE, France)



more rods than cones (Jonas *et al.*, 1992). These values differ according to species. In pigs, highest rod and cone densities were 153,000 and 39,000/mm<sup>2</sup>, respectively, and in sheep, 270,000 and 28,000/mm<sup>2</sup>, respectively (Chandler *et al.*, 1999; Shinozaki *et al.*, 2010). Rod to cone ratios ranged from 3:1 to 16:1 in pigs and from 6:1 to 20:1 in sheep (Chandler *et al.*, 1999; Shinozaki *et al.*, 2010). In birds, the ratios are very different. Common birds of various species had 55,000 rods and 175,000 cones/mm<sup>2</sup> (McNeil *et al.*, 2005).

Human acuity is exceptionally high compared with most animal species (Caves *et al.*, 2018). Visual acuity depends on the number of cones, but also many other factors, such as by how retinal circuits process the information (Caves *et al.*, 2018). Furthermore, many mammalian species (with the exception of primates, pigs, and rodents) have a tapetum, a reflective layer located behind the retina. By reflecting visible light back through the retina, it increases the light available to the photoreceptors facilitating vision under low light conditions. However, the tapetum may cause blur under daylight conditions (Maggs *et al.*, 2008).

Humans have three types of cones which allow the detection of different wavelengths, that is, colours, of light. The three types have peak wavelengths in the range of 564-580 nm, 534-545 nm, and 420-440 nm, perceived as red, green and blue, respectively. The difference in the signals received from the three cone types allows the brain to perceive a continuous range of colours. The context dependency of colour perception is shown by laboratory studies indicating that exactly the same wavelength may be perceived differently depending on its surround (Purves *et al.*, 2004).

Most mammals have only two types of cones. Pigs, sheep, goats and cows have cones with spectral peaks of 439-455 nm and 552-556 nm (Jacobs *et al.*, 1998; Neitz and Jacobs, 1989). Birds have four types of cones, with one type allowing the perception of very short wave lengths, towards the ultraviolet. Overall, this means that farm animals do not have the same colour perception as humans. Sheep were able to distinguish colours of wavelengths from 520-640 nm (Alexander and Stevens, 1979). A study on colour discrimination in cattle found that 5 out of 8 heifers distinguished red from blue and only three distinguished blue from green and green from red (Gilbert and Arave, 1986). Other studies gave similar results (Dabrowska *et al.*, 1981; Riol *et al.*, 1989). However, such studies do not give a complete image of the complex interactions between outputs of the photoreceptors (Jacobs, 1992, 1993). In addition, different colours have also different brightness that may be used as cues for the animals, rather than wavelength (Jacobs, 1993).

The eye needs to adapt to changes in light conditions. The adjustment of the aperture of the pupil is a fast response to adjust the amount of light that reaches the retina. However, its contribution to the overall light adaptation response is very small. An important mechanism is the intra-cellular response of the rods to incoming photons, which is adjusted to the light conditions of the environment. Furthermore, the retina contains a vast network of horizontal cells of which the activity depends on the brightness of the surrounding illumination. These horizontal cells are thought to influence the responsiveness of the photoreceptors to incoming photons (Miller and Tredici, 1992).

The above mechanisms play an important role during light adaptation: when suddenly the environment becomes much brighter, the eye has to adapt quickly to increased background illumination and be capable to discern objects in this brighter background. In humans, light adaptation occurs within seconds (Miller and Tredici, 1992). Dark adaptation, where the eye needs to adjust to very low light following bright light conditions, is in contrast a much slower process. Although the above cellular and neural adjustments occur within seconds, they are not sufficient. For complete dark adaptation, rods and cones need to regenerate their photosensitive pigments. In humans, the first phase of dark adaptation, based on the regeneration of pigments of cones, takes 5 to 8 min. The recovery of rod sensitivity needs much more time: about 40-50 min for completion (Reuter, 2011).

The pupillary light reflex, that is, the rapid constriction of the pupil in response to bright light, takes less than a second in humans, but several seconds in horses, cattle and sheep (Hachol *et al.*, 2006; Maggs *et al.*, 2008). In addition, as most non-human animals have a lower cone/rod ration than humans, they have also a less efficient dark adaptation (Wouters *et al.*, 1980). Little is known of the reactivity of retinal intracellular and neural mechanisms involved in the

adaptation to surrounding illumination in animals. However, behavioural responses of animals to changing light conditions suggest that the retinal responses are relatively slow (Grandin, 2019; Willson *et al.*, 2021).

The organisation of the eye is adapted to the habitat and characteristics of the species. For example, herbivores have horizontally elongated pupils. The pupils remain more or less horizontally orientated through a rotation movement of the eyeball, including when the animals are grazing (Banks *et al.*, 2015). In sheep and probably other herbivores, the horizontal pupils, the lateral placement of the eyes, and the specific distribution of cones, rods and the tapetum, allow the creation of sharp images of horizontal contours ahead and behind, creating a horizontally panoramic view that facilitates detection of predators from various directions, including when grazing at low light conditions, and forward locomotion across uneven terrain (Banks *et al.*, 2015; Piggins and Phillips, 1996; Shinozaki *et al.*, 2010). Summarising, 'seeing' is a perception and not a faithful rendition of the external world in all its factual detail. The visual system takes great liberties with the information it receives from the external environment and uses a number of shortcuts to process the image rapidly into information relevant to the species so that can be used to guide behaviours (Gilger, 2010). Hence, what is perceived by a normal, human eye does not necessarily reflect what is perceived by the eye of the various farm animal species.

Similarly, hissing or high frequency sounds may seem unimportant or simply remain undetected by humans (Heffner and Heffner, 1992; Phillips, 2010; Box 1.7), whereas they may induce fear-responses in animals (Grandin, 2019).

### **Box 1.7. Sound perception in farm animals.**

Cattle perceive sounds up to around 35 kHz compared to about 20 kHz for humans (Adamczyk *et al.*, 2015; Heffner and Heffner, 1992). The human ear is most sensitive to sounds ranging between 500 to 4 kHz (Weeks *et al.*, 2009). The low frequencies hearing limit of cattle is similar to that of humans (about 25-30 Hz; Adamczyk *et al.*, 2015; Phillips, 2010). Thresholds for discomfort for cattle were noted at 70-85 dB, with physical damage to the ear occurring at 110 dB (Adamczyk *et al.*, 2015; Phillips, 2010; Weeks *et al.*, 2009). The auditory range of sheep is 125 to 40 kHz and for pigs between 42 and 40 kHz (Adamczyk *et al.*, 2015; Brouček, 2014; Weeks, 2008). Pigs show stress responses to sounds above 70 dB (Goumon and Faucitano, 2017). Loud (around 100 dB) and sudden noise induce stress responses in animals during the slaughter period. For example, the shot of the captive bolt device used to stun cattle produces sounds of 110 dB and is perceived by the other animals in the slaughter corridor resulting in stress (Gregory *et al.*, 2007).

### 1.2.3 Physiological stress responses that can be measured in the preslaughter and slaughter context

#### 1.2.3.1 Metabolic indicators

The preslaughter period is a physically and psychologically demanding situation, which needs energy expenditure to allow increased physical action and vigilance. In addition, food deprivation is generally practised to avoid full intestines at slaughter because during carcass dressing, it reduces the risk to open accidentally the filled digestive tract, which would lead to microbial contamination

of the carcass. In pigs, food deprivation reduces further travel sickness, during transport, expressed as sniffing, foaming and chomping and retching and vomiting (Bradshaw *et al.*, 1996; Faucitano and Raj, 2022 – Chapter 5 of this book; Rioja-Lang *et al.*, 2019). The food deprivation, physical activity and psychological stress all influence the metabolism of the animals.

The two major energy substrates of the body are glucose and free fatty acids (FFA). Glucose is a monosaccharide; it is stored as a multi-branched polysaccharide, called glycogen, in skeletal muscle and the liver. FFAs are elongated hydrocarbon chains with a terminal carboxylate group and are stored in body fat. Glucose and FFA need to be degraded via specific pathways to yield energy. The first step of glucose breakdown is called glycolysis and does not require oxygen. It yields pyruvate, which, if sufficient oxygen is present, enters the mitochondrion for a subsequent process called oxidative phosphorylation (Robergs *et al.*, 2004). Both glycolysis and oxidative phosphorylation yield adenosine triphosphate (ATP), an energy carrying organic compound necessary for processes that would not occur automatically such as metabolic reactions, transport of substances across membranes, nerve impulse propagation and muscle contraction. Their efficiency is very different: glycolysis yields 2 ATP per glucose, much less than the oxidative phosphorylation that yields about 30 ATP (Robergs *et al.*, 2004).

FFA are broken down via  $\beta$ -oxidation and oxidative phosphorylation in the mitochondria to yield ATP. The amount of ATP the FFA breakdown yields depends on the length of the chain of the fatty acid, but FFA are very rich in energy. For instance, palmitic acid, a common FFA in the body, contains 16 carbon atoms, and yields 106 ATP (Darvey, 1998).

The availability of glucose and FFA in the plasma and in organs are tuned together. Under basal circumstances, glucose is the primary energy source for skeletal muscle. Food deprivation diminishes plasma glucose levels and glycogen contents in the liver and muscle (Knowles *et al.*, 1999). When glucose levels are relatively low or when needs are high, plasma FFA levels, and their uptake and oxidation by the muscles increase (Romijn *et al.*, 1995). The elevation of plasma FFA levels further stimulates glycogenolysis (breaking down of glycogen into glucose) and hepatic gluconeogenesis (formation of glucose from lactate, pyruvate, glycerol and amino acids), thus enhancing availability of glucose (Lam *et al.*, 2003; Staehr *et al.*, 2003). The maintenance of glucose levels is of major importance for the functioning of the brain, which uses essentially glucose as energy source, contrary to other organs (Kuo *et al.*, 2015). Ruminants are capable of forming  $\beta$ -hydroxybutyrate from volatile fatty acids that are produced by rumen fermentation and use it as alternative source of energy.

Due to the increased effort, plasma glucose and FFA levels change during the preslaughter period (Table 1.5). For example, during transport, plasma levels of FFA and glucose increased (Jarvis *et al.*, 1996a; Knowles *et al.*, 1999; McVeigh and Tarrant, 1982; Warriss *et al.*, 1995). Following long transports, a decrease in glucose may also be observed (Navarro *et al.*, 2019). In this case, the consumption of glucose outweighed the capacity of replenishment, probably due to depletion of liver and muscle glycogen reserves. In agreement, durations of more than 14 h of transport and food deprivation led to lower glycogen contents in the muscle (Knowles *et al.*, 1999). Following transport by road or sea, food deprived cattle had higher plasma  $\beta$ -hydroxybutyrate levels (Table 1.5; Navarro *et al.*, 2019; Warriss *et al.*, 1995). The increase in  $\beta$ -hydroxybutyrate may reflect increased needs,

**Table 1.5.** Indicators related to physical condition in meat animals.

Variable	Cause
Glucose (plasma)	physical effort, food deprivation, stress
Free fatty acids (plasma)	
Lactate (whole blood, plasma, muscles, liver)	
Urea (plasma)	food/water deprivation
Total proteins (plasma)	
Albumin (plasma)	
Haematocrit/packed cell volume (plasma)	
$\beta$ -hydroxybutyrate (plasma)	
Creatine kinase (plasma, serum, muscle)	physical effort
$\beta$ -endorphins (plasma)	stress
$\beta$ -endorphins (pain matrix)	pain

associated or not with reduced glucose availability. In keeping with this, the increased levels of  $\beta$ -hydroxybutyrate were accompanied by increased levels of glucose and FFA in one study (Warriss *et al.*, 1995) and by a decrease in glucose levels in the other (Navarro *et al.*, 2019).

The increased production of ATP in the skeletal muscle is necessary to allow muscle contraction. This involves binding of myosin heads to strings of actin, which is an energy-needing process. The energy is delivered by the hydrolysis at the myosin head of ATP into adenosine-di-phosphate (ADP). Creatine kinase (CK) plays an essential role in maintaining ATP levels by catalysing the transfer from creatine phosphate to the ADP to yield ATP. Plasma CK levels increase following vigorous or long-lasting physical effort, due to damage of or a transient increase in the permeability of the muscle fibre membranes (Baird *et al.*, 2012; Ferraz *et al.*, 2010; Warriss *et al.*, 1995). The time of CK release into and clearance from plasma depends on the level of training (or daily exercise), type, intensity and duration of exercise, age and gender (Brancaccio *et al.*, 2007). For example, post-exercise plasma CK levels were higher in men than women (Sewright *et al.*, 2008). Peak plasma CK values depend on the type of exercise. Eight hours after strength training (such as lifting light or moderate weights), they are about two-fold above baseline (Brancaccio *et al.*, 2007). Eccentric exercise (a slow muscle elongation exercise) causes a pronounced increase only between 2 and 7 days after exercise, due to the muscle damage associated with the exercise (Brancaccio *et al.*, 2007). After prolonged exercise, total serum CK activity is markedly elevated for 24 h after the exercise bout when subjects rest and remain elevated for longer in the absence of rest (Brancaccio *et al.*, 2007).

Blood CK levels were increased following transport in pigs and cattle (Table 1.5; Knowles *et al.*, 1999; Śmiecińska *et al.*, 2011; Warriss *et al.*, 1995) and following thermal stress in poultry (Debut *et al.*, 2005; Mitchell and Sandercock, 1995; Mitchell *et al.*, 1992). Compared to cattle arriving directly from the farm, plasma CK activity in blood collected at exsanguination was significantly greater in cattle from markets, probably caused by additional handling and transport (Jarvis *et al.*, 1996a). Pigs transported in a top rear compartment of the lorry or for a longer duration (12 or 18 h) had higher levels of serum CK than those transported in a top front compartment or for a shorter duration (6 h; Somavilla *et al.*, 2017). Plasma CK levels were higher in exsanguination blood in

abattoirs considered particularly stressful for pigs and after transport and slaughter described as having a low welfare index (Sardi *et al.*, 2020; Warriss *et al.*, 1994). In certain experiments, serum CK levels following transport were higher in winter than in summer (Correa *et al.*, 2013, 2014). Other experiments found the opposite (Sommavilla *et al.*, 2017). Lower increases during the warmer season may be explained by lower activity levels due to high ambient temperatures (Nybo *et al.*, 2013).

Lactate is a product of glycolysis when levels of oxygen in the cells are low. Under normal conditions, in humans, lactate is produced at a rate of about 15-30 mmol/kg/day. Blood lactate levels are maintained between 0.5 and 1.0 mmol/l, which reflects the balance between lactate production and consumption (Allen and Holm, 2008). In the exercising muscle, oxygen levels are generally insufficient, and consequently, plasma lactate levels may rise to levels above 20 mmol/l. The effort-related rise is only transitory because the body has the capacity for rapid lactate disposal, which at least in humans can rise to 500 mmol/h. In the resting state, the liver and kidneys account for disposal of around 60 and 25-30% of circulating lactate by converting lactate into glucose (Cohen and Woods, 1983). During exercise at higher ambient temperatures, lactate production may be greater (Claremont *et al.*, 1975; Morrissey *et al.*, 2019), but opposite effects have also been reported (Marino *et al.*, 2001).

The above indicates that plasma lactate levels depend on many factors, such as the oxidative capacity of the muscles, the level of daily exercise of the animal, the ambient temperature and the capacity of the liver and kidneys to convert lactate into glucose (Thomas *et al.*, 2004). However, overall, during transport and slaughter, plasma lactate levels rise due the physical activity associated with this period (Brandt and Aaslyng, 2015; Table 1.5). Greater levels of lactate were observed in pigs loaded on steep ramps or using electrical prods, and following longer durations of transport, compared to controls (Faucitano and Lambooj, 2019). Pigs showing more often rearing, walking backwards and being more often involved in jams while attempting to enter a space had higher lactate levels (Edwards *et al.*, 2010). Seasonal effects were also observed and seemed to be associated with seasonal effects on activity levels. Specifically, one study found greater (Correa *et al.*, 2013), and another smaller lactate increases during transport and lairage in summer compared to winter (Correa *et al.*, 2014). These differences reflected higher and lower activity levels in summer compared to winter, respectively (Correa *et al.*, 2013, 2014).

Plasma lactate levels and body temperature both rise during exercise (Claremont *et al.*, 1975). Accordingly, ocular temperature, measured by infrared thermography and being an indicator of core temperature, was positively correlated, albeit weakly, with plasma lactate levels (Weschenfelder *et al.*, 2013). However, in transported pigs, negative correlations were found between plasma lactate levels on the one, and temperature in the gastrointestinal tract on the other hand (Sommavilla *et al.*, 2017). The reason for this negative association remains to be explained.

### 1.2.3.2 Body fluids

Body fluids are often considered according to their fluid compartment. The intracellular fluid compartment refers to all fluids enclosed in cells by their plasma membranes. It represents about

two thirds of all body fluids. Extracellular fluid represents one third and is constituted of the fluid component of the blood and the interstitial fluid that surrounds all cells. Dehydration causes increases in blood osmolality, in plasma levels of urea, a waste product of protein breakdown, in total proteins, in albumin, one of the most abundant proteins in the blood and involved in carrying certain hormones, and in packed cell volume (PCV), a measurement of the percentage of blood that is made up of cells (Jarvis *et al.*, 1996a; Knowles *et al.*, 1999; Navarro *et al.*, 2019; Warriss *et al.*, 1995). Normal reference PCV values are 24-46, 36-43 and 27-45% for cows, pigs and sheep, respectively (Latimer, 2011; Weiss and Wardrop, 2010). Haematocrits (red blood cells) account for nearly all the cells in the blood. Under stressful conditions, such as during transport and restraint in the box prior to religious bleeding, the spleen may release haematocrits into the blood stream, which may be another cause of an increase in PCV (Bourguet *et al.*, 2011b; Liotta *et al.*, 2007; Warriss *et al.*, 1995). It is useful to combine several indicators, physiological and behavioural, to evaluate a possible state of dehydration. Only plasma total protein concentration in blood collected at exsanguination was significantly greater in cattle arrived from markets than in those from farms (Jarvis *et al.*, 1996a). Cattle from distant markets had higher PCV and plasma total protein concentration than those from markets at shorter distances, indicating additional effects of transport duration on dehydration. The cattle from markets spent more time drinking during the initial 3 h in the lairage pen, in agreement with their state of dehydration (Jarvis *et al.*, 1996a). In pigs, plasma albumin levels were greater after unloading than before loading (Averos *et al.*, 2007). The clinical status of the animal must be taken into account as various diseases may also influence haematological indicators (Abutarbush, 2015).

The combination of food and water deprivation may result in decreased water in extracellular space, different body compartments and in Na excretion in urine in sheep and cattle, indicative of an imbalance of the normal water and electrolyte distribution (Cole, 2000; Hogan *et al.*, 2007).

### 1.2.3.3 Nervous system and hormones

The changes in metabolism described above are associated with other physiological responses that allow an increase in availability of energy substrates, efficient gas exchanges and increased blood circulation to the organs that are most needed for the increased activity and vigilance needed in demanding situations. These responses involve changes in neurological and endocrine systems. They may be induced by psychological stress, without any overt behavioural reaction, but also by physical activity, without any psychological stress. The combined physiological responses help sustain the increased physical effort and vigilance needed in stressful situations. Certain responses occur very fast, other changes occur progressively over the time of the duration of the stressor or of the increased activity. Generally, the physiological responses are more or less proportional to the stress level of the animal, until the system reaches a certain ceiling threshold.

The autonomic nervous system is a neurological system involved in vital functions, consisting of a sympathetic and a parasympathetic branch, which have opposite effects. Their balance has a direct influence on the activity of many organs, the sympathetic branch stimulating physically active responses, while the parasympathetic stimulates body functions associated with resting and digesting. During psychological stress and physical activity, the sympathetic/parasympathetic balance shifts towards increased sympathetic activity. It is a fast responding system. Increased

sympathetic activity causes the release of catecholamines (i.e. adrenaline, noradrenaline and dopamine) from the medulla of the adrenal glands. However, most of the noradrenaline and dopamine present in the blood stream has diffused from the noradrenergic nerve endings of the sympathetic nervous system into the blood (Goldstein and Holmes, 2008). The role of dopamine in acute stress responses is not well understood. Increased activity of the sympathetic nerves to the heart and increased adrenaline levels accelerates heart rate and increases stroke volume, while blood pressure rises due to vasoconstriction, providing better perfusion of the vital organs and muscles. Sympathetic activity stimulates the dilation of bronchioles, hence facilitating gas exchanges in the lungs and decreases the variability between inter-beat intervals of the heart, resulting in lower heart rate variability (Shaffer and Ginsberg, 2017). Low heart rate variability can therefore be used as an indicator of acute stress (Després *et al.*, 2003). Increased sympathetic activity further relaxes accommodation and dilates the pupils, letting more light into the eyes, and constricts the blood vessels of the skin, which limits bleeding from wounds and makes more blood available for organs. It also stimulates structures in the brain stem, particularly the reticular formation, responsible for vigilance. The shift towards the stronger sympathetic and lower parasympathetic input continues as long as the threat is perceived. Removal of the stress is associated with a relatively rapid restoration of the pre-stress balance.

Stress causes further the activation of the hypothalamic-pituitary-adrenal axis (HPA). This axis is controlled by neurons in the hypothalamus of the brain, secreting the corticotropin releasing hormone. This hormone stimulates the secretion of adrenocorticotrophic hormone (ACTH) into the bloodstream from the anterior lobe of the pituitary. The ACTH subsequently reaches the adrenal cortex via the blood stream where it stimulates the secretion of glucocorticoids (i.e. cortisol in most mammals and corticosterone in rodents, birds, reptiles and amphibians). The HPA is a relatively slowly responding system, often reaching (context-dependent) maximal values after 30 to 60 min after onset of the stress (Herman *et al.*, 2016). Plasma cortisol has a half-life of 66 min at normal hormone levels (Weitzman *et al.*, 1971). Consequently, increased cortisol may still be observed after removal of the stress. When pre-stress levels are restored it is considered that the animals have completely recovered from the stressful event (Knowles *et al.*, 1999).

Catecholamines and glucocorticoids act in concert. The presence of glucocorticoids is necessary to allow the effect of catecholamines on the vasoconstriction of blood vessels (Barrett *et al.*, 2016). Increased circulating glucocorticoid and catecholamine concentrations contribute to mobilise energy substrates, glucose and FFA, in the blood. More specifically, adrenaline stimulates lipolysis and glycogenolysis in the liver and muscle (Qvisth *et al.*, 2006; Watt *et al.*, 2001). Hence, during stress, the combined increase in cortisol and adrenaline make lipids available from adipose tissue and glucose through glycogenolysis for increased muscle activity, while energy storage is inhibited. Glucocorticoids block further insulin-mediated glucose uptake by the muscles reserving part of the blood glucose for brain functioning (Bingham *et al.*, 2002; Kuo *et al.*, 2015).

Given their prominent roles in adaptive responses, the autonomic nervous system and HPA axis are strongly influenced by pre-slaughter procedures. Birds being caught and carried upside down (Duncan *et al.*, 1986; Kannan and Mench, 1996) and adult cattle, calves, sheep and pigs after transport, loading and unloading showed increased heart rates and glucocorticoids (Baldock and

Sibly, 1990; Bradshaw *et al.*, 1996; Kenny and Tarrant, 1987a,b; Knowles *et al.*, 1999; Śmiecińska *et al.*, 2011; Van de Water *et al.*, 2003). Increased plasma levels of adrenaline and glucocorticoids have been reported in fighting pigs (Fernandez *et al.*, 1994; Terlouw *et al.*, 2005) and in fish, under conditions of crowding, handling, struggling or hypoxia (Barton, 2002; Mazeaud *et al.*, 1977; Ramsay *et al.*, 2006). Following a long (14 to 31 h) transport, cattle that lay down in lairage had higher plasma glucocorticoids levels than those that remained standing (Knowles *et al.*, 1999), indicating a relationship with level of fatigue. Low welfare levels of transport and slaughter in pigs were associated with higher cortisol and adrenaline levels (Sardi *et al.*, 2020). Introduction into the stunning box or the restrainer in cattle and pigs were associated with faster heart rates (Chevillon, 2000; Knowles, 1999; Reiche *et al.*, 2019).

The measurement of physiological indicators of pain is relatively complex. Pain induces physiological changes that are similar to non-painful responses to stress (Prunier *et al.*, 2013). The pain perceived can be modulated by  $\beta$ -endorphins in humans or in animals (Box 1.8). Some reports mention the possibility that  $\beta$ -endorphins are secreted following bleeding without prior stunning (EFSA, 2004). Today, there are little arguments to support this. Although a study in sheep found an increase in the level of  $\beta$ -endorphins in the blood following a slow haemorrhage, compared to a control group, this increase was gradual until reaching a maximum value only after 45 min (Smith *et al.*, 1986). Furthermore, a later cattle study reported that blood endorphin levels did not increase after bleeding without stunning (Zulkifli *et al.*, 2014). Overall, there is much doubt on a role of circulating endorphins in acute pain perception (Box 1.8).

### Box 1.8. The role of endorphins in pain.

In humans and animals, the secretion of endogenous opioid peptides, called  $\beta$ -endorphins, can reduce the pain experienced. Endorphins act on opiate receptors and have analgesic action at all levels of the pain system: at the level of the wound, the spinal cord and the brain (Luan *et al.*, 2017).

The role of  $\beta$ -endorphins in the central nervous system in pain perception has been clearly established (Millan *et al.*, 1987). The concentration of  $\beta$ -endorphins in the brain increases rapidly after noxious stimulation, which demonstrates its role as a regulatory mechanism (Young *et al.*, 1993). Micro-injections of morphine (a synthetic opioid) into certain areas of the pain matrix decrease, and of agents blocking the effects of  $\beta$ -endorphins increase, pain-related behaviours in rats (Basbaum and Fields, 1984).

The role of  $\beta$ -endorphins present in the blood in pain perception is less clear. The pituitary gland secretes  $\beta$ -endorphins into the bloodstream in response to stress or pain. In production animals, stressful or painful procedures, such as shearing, electro-immobilisation or tail-docking in sheep, provoked  $\beta$ -endorphins secretion in blood (Jephcott *et al.*, 1987; Shutt *et al.*, 1987). However, these increases in plasma  $\beta$ -endorphins are believed to be a response to stress rather than acute pain (Bach *et al.*, 1987). Circulating endorphins do not seem to reach the central nervous system and the level of pain perceived is generally not correlated with plasma  $\beta$ -endorphin levels (Basbaum and Fields, 1984; Johansen *et al.*, 2003). Basal circulating  $\beta$ -endorphin levels may, however, be involved in chronic pain perception (Bruehl *et al.*, 2012; Falcone *et al.*, 1988).

Finally, the immune system produces  $\beta$ -endorphins to reduce inflammatory (non-acute) pain, resulting from healing processes. These  $\beta$ -endorphins are produced and secreted by immune cells locally present in inflamed tissues, for



example in wounds, and act on primary nociceptors (Stein, 2013). The endorphin receptors are produced by the nociceptor itself, at the level of the dorsal root, and transported to the wound site (Mousa *et al.*, 2001).

The secretion of endorphins in certain brain structures are in contrast associated with pain perception (Box 1.8). However, in induced haemorrhages in sheep, the concentration of  $\beta$ -endorphins in the cerebrospinal fluid was not significantly different from that of non-haemorrhaged sheep (Smith *et al.*, 1986). The possibility of an increase in the brain or the increase of other molecules of this opioid family, such as enkephalin or dynorphin, remains to be studied.

#### 1.2.3.4 Sampling

Saliva and blood samples may be collected during the pre-slaughter period or just after stunning to evaluate blood constituents (Table 1.5 and 1.6). Blood samples may be taken directly from the jugular vein (venepuncture) in the case of cattle and sheep. For pigs, blood can be sampled from the ear vein or using an indwelling jugular catheter. In pigs and sheep, bleeding takes place immediately after stunning leaving no time allowance for sampling, but blood can be sampled from the bleeding wound. This is less precise as the sample obtained is a mixture of arterial and venous blood, which differ in levels of plasma constituents. In addition, the stunning procedure, particularly electrical stunning may influence hormone levels, such as catecholamines (Shaw and Tume, 1990). After slaughter, urine may be sampled from the bladder to estimate catecholamine (adrenaline, noradrenaline and dopamine) and glucocorticoids (cortisol, corticosterone) levels (Bourguet *et al.*, 2015). Plasma hormone levels reflect the physiological status on the moment of sampling, while because of their slower diffusion, the hormones levels in the saliva and urine reflect the physiological status of the animal over a longer period (Elfering *et al.*, 2003; Jung *et al.*, 2014).

### 1.3 Consequences of stress reactions on carcass and meat quality

#### 1.3.1 Carcass quality

Certain animal stress indicators can be collected at the levels of the carcass. Food and/or water deprivation obviously result in weight loss (Knowles, 1999; Nicol and Scott, 1990). After slaughter, the carcass can be inspected for different types of skin damage, caused by human-animal, animal-animal and animal-facility interactions (Strappini *et al.*, 2013). Bruises result from damaged blood vessels due to a blow on the skin, and scratches are surface wounds. They may be caused by the use of sticks, collisions with equipment or other animals, or by a vertical sliding door closed while an animal is underneath, striking its back or shoulders (Bourguet *et al.*, 2011a; Sanchez-Hidalgo *et al.*, 2020; Strappini *et al.*, 2013). Lacerations and tears penetrate the skin and are caused by sharp objects, such as hoofs and teeth of conspecifics, or sharp pen fittings. The part of the carcass may also be distinguished, such as the head and shoulders, ears, middle part and hind part. Certain events cause more specifically lesions on specific parts of the body. For example, fighting pig direct their bites most often, but not exclusively, to the front parts (head, should, ears) and vertical sliding doors impact the back and shoulders of cattle (Bottacini *et al.*, 2018; Strappini *et al.*, 2013). Pigs mounting other pigs scratch with their forelegs the back of the mounted pig (Faucitano, 2001). During the

**Table 1.6.** Examples of physiological responses to stress and meat quality aspects that are influenced by stress, relative to the preslaughter context (Section 1.2.3, 1.3.1 and 1.3.2).

Variable indicative of stress	Interpretation in terms of effects of stress
<b>Ante-mortem</b>	
Fast heart rate Low heart rate variability	Immediate response to stress of psychological origin, and/or to physical effort
High adrenaline and noradrenaline levels (plasma, saliva, urine)	The substances are secreted in the blood in response to stress, and progressively enter saliva and urine
High cortisol levels (plasma, saliva, urine)	Progressive response to stress of psychological origin, and/or to physical effort. The substances are secreted in the blood, and progressively enter saliva and urine
<b>At bleeding</b>	
High plasma adrenaline and noradrenaline levels	Immediate response to stress of psychological origin, and/or to physical effort
High plasma cortisol levels	Progressive response to stress of psychological origin, and/or to physical effort
<b>Post-mortem</b>	
High urinary adrenaline and noradrenaline levels <sup>1</sup> High urinary cortisol levels <sup>1</sup>	Indicative of ante-mortem stress of psychological origin, and/or to physical effort
Low early post-mortem pH <sup>1</sup> Light meat colour <sup>2</sup> High drip loss <sup>3</sup>	Indicative of stress just before stunning/bleeding
High pHu <sup>2</sup> Dark meat colour <sup>2</sup> Low drip loss <sup>3</sup> Low muscle glycogen content <sup>4</sup>	Indicative of stress during the pre-slaughter period
Carcass lesions	Aggressive behaviour, interventions (slaps, sticks) by humans, impacts from equipment
Decreased tenderness or juiciness	Stress during the preslaughter period. The exact mechanisms vary according to the moment of stress and the species. For pigs, the underlying mechanism is to a certain extent explained by rate and extent of pH decline. For cattle, the underlying mechanisms are not well known

<sup>1</sup> Generally measured between 30 and 60 min post-mortem.

<sup>2</sup> Generally measured around 24 h post-mortem.

<sup>3</sup> Generally measured over several days post-mortem.

<sup>4</sup> Glycogen is progressively degraded over the first 24 h post-mortem. The interpretation depends on the moment of sampling.

resolution of a bruise, generally, its colour changes from red or purple to blue, then to brown, yellow or green (Bohnert *et al.*, 2000; Northcutt *et al.*, 2000). Characteristics of tissue injured, mechanism of injury, length, duration of force, and depth of injury amongst other influence the speed of this process (Bohnert *et al.*, 2000; Northcutt *et al.*, 2000). Generally, bright red bruises have occurred more recently than darker coloured bruises (Faucitano, 2001), although in fowl, the changes may go in the opposite direction in certain muscles (Northcutt *et al.*, 2000). A precise timing using

visual colour assessment is difficult, however. A detailed analysis of skin lesions caused by biting or mounting found that recent wounds (<7 h) were darker and redder than older wounds (>7 h) when assessing the colours spectrophotometrically, but visual assessment of the colours did not allow this differentiation accurately (Vitali *et al.*, 2017).

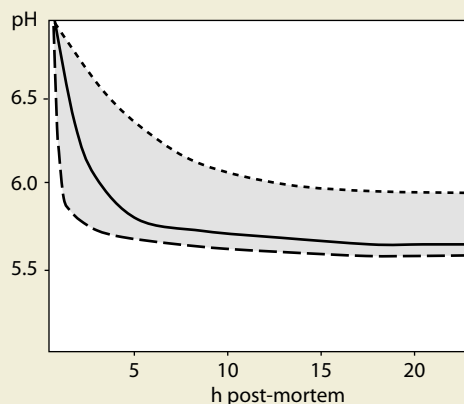
Skin damage may occur at varying moments of the slaughter period, during transport, lairage and in the stunning box, but may also already have been present before leaving the farm. If injuries are present irrespectively of the transporter or farm, it may be assumed that they have occurred at the abattoir and animals need to be observed during their trajectory through the abattoir to identify the risk zones. In cattle, bruising was largely related to animal-facility and human-animal interactions (Strappini *et al.*, 2013). However, animal-animal interactions can lead to very severe conditions with fallen cattle being stamped on by other animals (Bourguet *et al.*, 2011b; Strappini *et al.*, 2013). In pigs, increased on-farm fasting time, mixing of unfamiliar groups, longer loading times, increased human interventions during loading and greater space allowance during transport were associated with increased amounts of scratches and lacerations (Correa *et al.*, 2013; Guàrdia *et al.*, 2009). Body weight or age, number of falls and slips due to poor handling or poor equipment and overall level of activity may also influence degree of skin damage in cattle and pigs (Correa *et al.*, 2010; Grandin, 1980; Mpakama *et al.*, 2014; Tarrant *et al.*, 1992). Electrical goads do not mark the skin, unless they are vigorously applied on the animal, but may indirectly lead to increased skin damage due to the increased speed of pigs, frequency of slips, and panic-like reactions that make them collide with walls and other objects (Correa *et al.*, 2010; McGlone *et al.*, 2004; Rabaste *et al.*, 2007; Strappini *et al.*, 2013). Similar observations were made for cattle (Jarvis *et al.*, 1995). In pigs, increased mounting and associated damage was caused by overcrowding (Faucitano, 2001). Seasonal effects are sometimes observed with higher degrees of skin damage during certain seasons, but the effects seem generally indirect, and related to other factors, such as different weights, handling practices, transport conditions or activity levels, which vary according to season (Correa *et al.*, 2013; Dalla Costa *et al.*, 2007). In cattle, interventions that potentially cause bruises often leave no visible bruise (Jarvis *et al.*, 1996; Strappini *et al.*, 2013). This indicates that the indicator is relatively insensitive and that negative events may have occurred without leaving marks on the skin.

In birds, the most frequent injuries seen are bruises, broken limbs and damaged wings (Nicol and Scott, 1990). Damaged wings are often caused by wing flapping, which may cause bruising, dislocation, broken wings, and haemorrhage resulting in red wing tips after slaughter (Gregory, 1994; Gregory *et al.*, 1989; Knowles and Broom, 1990; Lambooij *et al.*, 2010; Mitchell and De Boom, 1986; Rao *et al.*, 2013). Wing flapping and struggling may result from manual catching, lifting, holding, and carrying the birds inverted by their legs. Wing flapping may also occur after placing them in the transport crates or after shackling them on the slaughter line (Gerritzen *et al.*, 2022 – Chapter 6 of this book). Joint dislocations and haemorrhaging may result from carrying birds by one leg, amongst others, during loading (Cockram *et al.*, 2020; Nicol and Scott, 1990). Significantly more fractures occurred during pre-slaughter handling at the abattoirs than during catching/transportation (Kittelsen *et al.*, 2015). The authors stress that despite their lower prevalence, damage occurring before arrival at the abattoir are of particular importance, due to the longer duration of suffering (Kittelsen *et al.*, 2015). Factors that influenced the risk of wing injuries were the handlers, weight of the birds, number of birds per cage, loading speed, season and time of day (Cockram

*et al.*, 2020). Care must be taken with interpretation of the data obtained on the carcass, because damage caused by stunning, whether electrical or by CO<sub>2</sub>, is not a welfare issue as the animals are unconscious when it occurs, provided that the stunning process is carried out correctly. Thus, CO<sub>2</sub> stunning may cause wing fractures, due to struggling, wing flapping and muscular spasms experienced during the last phase of the stunning in unconscious birds (Abeyesinghe *et al.*, 2007; McKeegan *et al.* 2007a,b). High current intensity stunning increases the incidence of red wing tips, bones fractures, dislocations, and haemorrhaging (Gregory and Wilkins, 1989). Similar effects may be observed in pigs where electrical stunning may induce bone fractures in the thoracic vertebrae and in the scapula where it joins the humerus (Channon *et al.*, 2003).

### 1.3.2 Meat quality

Pre-slaughter physiological and behavioural reactions of the animal influence ante- and post-mortem muscle energy metabolism with potentially very negative effects on the quality of meat (Bendall, 1973; Bourguet *et al.*, 2015; Hambrecht *et al.*, 2005; Rocha *et al.*, 2016; Terlouw and Rybarczyk, 2008). Following bleeding of the animal, biochemical reactions continue in the muscle cells for several hours. These reactions are necessary for the conversion of muscle into meat. The reactions are not identical to those occurring in the living animal. Since blood no longer circulates, glucose and oxygen are not delivered to the muscle. The reactions use therefore glycogen stored locally in the muscle as energy source. Due to the absence of oxygen, glycogen is catabolised anaerobically, causing a net production of hydrogen ions (H<sup>+</sup>), amongst others. Due to the absence of blood circulation, these ions accumulate in the muscle resulting in a pH decline (Hambrecht *et al.*, 2005; Robergs *et al.*, 2004). This decline is initially fast, then slows to reach a stable value of around 5.5, reached approximately 24 h post-mortem, referred to as the ultimate pH (pHu). The rate and extent of the pH decline may have significant effects on the quality of the meat that is produced (Figure 1.2).



**Figure 1.2.** The post-mortem pH decline in muscles is initially fast then slows to reach a stable value around 5.4-5.6. Preslaughter stress may cause a fast pH decline (broken line) or a pH decline of insufficient amplitude (dotted line). The resulting meats are of lesser quality.

The effects of stress reactions on post-mortem muscle metabolism and, consequently, on meat quality, can be distinguished in two broad categories. As muscle glycogen stores fuel the post-mortem metabolic reactions resulting in acidification, low glycogen levels at the moment of slaughter may result in relatively high pHu values. Low muscle glycogen levels may occur if the day or hours before slaughter physical activity and psychological stress were high and have consumed glycogen stores from the muscles. For instance, food deprivation, long transports and physical interactions between mixed unfamiliar animals lead to depletion of muscle glycogen and increased pHu values (Knowles *et al.*, 1999; McVeigh and Tarrant, 1982; Warriss, 1990). At high pHu value, the physical state of the proteins is above their iso-electric point and associate with more water. The meat appears dark because the fibres are more tightly packed and its surface does not scatter light to the same extent as the more open surface of meat with a lower pHu value (Seideman *et al.*, 1984). Extreme cases characterised by a pHu value >5.9 are called dark cutting (DFD: dark, firm, dry) meat (Figure 1.2). Although high pHu value has positive effects on water-holding capacity and tenderness, flavour and shelf life are degraded at values above 6.1 (Bidner *et al.*, 2004). DFD meat can occur in all major meat species (Ponnampalam *et al.*, 2017). The effects are only visible if glycogen levels reach a certain, low threshold (55  $\mu\text{mol}$  glucose equivalents/g fresh muscle tissue; Terlouw *et al.*, 2021). Basal glycogen levels are generally much higher, which means that only longer-lasting or very high stress levels will influence pHu values.

If on the other hand stress reactions take place in the minutes preceding slaughter, whole body and particularly muscle metabolic activity is high at the moment of death. This high metabolic activity continues after death of the animal, causing a faster than normal pH decline during the early post-mortem period (Figure 1.2). Studies found that stress or exercise immediately before slaughter, due to the use of an electric prod (D'Souza *et al.*, 1998), intense physical activity (Rosenvold and Andersen, 2003), or fighting (Karlsson and Lundström, 1992) resulted in higher muscle temperature and lactic acid content and faster pH value decline. As the carcass remains relatively warm due to increased heat production, the denaturation of muscle proteins is accelerated (Aalhus *et al.*, 1998; Bendall, 1973; Hambrecht *et al.*, 2005). This leads to light coloured meats with low water-holding capacity (high drip loss). Proteolytic activity is reduced, slowing the tenderisation process (Barbut *et al.*, 2008). During cooking, the denatured proteins precipitate and become less soluble adding to the toughness (Hegarty *et al.*, 1963; Minelli *et al.*, 1995). Extreme cases, characterised by a pH <6.0 at 45 min post-mortem are designated as PSE (pale, soft, exudative) meat (Bendall and Swatland, 1988). A fast early post-mortem pH value decline occurs more often in white muscle fibres as they are fast contracting and have a greater potential for a fast increase in catabolism compared to red fibres. Therefore, this defect is mainly observed in pigs and poultry, who have muscles relatively rich in white fibres, but it exists also in other species, including cattle (Aalhus *et al.*, 1998).

Particularly the combination of physical activity and adrenaline (Christensen and Galbo, 1983) may influence meat quality (Table 1.7). Adrenaline stimulates muscle glycogen breakdown specifically in the exercising muscle, while it has little effect when the muscle is at rest (Febbraio *et al.*, 1998). In abattoirs, the muscles of the animals are mostly not at rest as driving the animals through the alleys and races of the abattoir are inherent to the slaughter procedure. If animals are, in addition, psychologically stressed (e.g. avoidance and escape responses), they may be even more active. The psychological stress and physical activation cause increases in adrenaline levels. If this occurs just

**Table 1.7.** Examples of correlations among behavioural and physiological responses, during the slaughter period or in reactivity tests conducted during rearing, and meat quality.

Variable 1	Variable 2	r (p) values	Animal type	Reference
Vocalisations in response to use of the prod in the abattoir handling area	Lactate (exsanguination blood)	0.23 (0.006)	Slaughter pigs	Edwards <i>et al.</i> , 2010
Occurrences of jamming of pigs during driving in the abattoir		0.24 (0.005)		
Creatine kinase (CK) (exsanguination blood)	Adrenaline (urine, post-mortem)	0.28 (<0.05)	Slaughter pigs	Foury <i>et al.</i> , 2011
CK (exsanguination blood)	pH 24 h post-mortem ( <i>Semimembranosus</i> muscle)	0.17 (<0.05)		
Adrenaline (urine, post-mortem)		0.44 (<0.001)		
Noradrenaline (urine, post-mortem)		0.31 (<0.05)		
Ocular infrared temperature	Lactate (blood sample in restrainer)	0.20 (0.001)	Slaughter pigs	Weschenfelder <i>et al.</i> , 2013
	pH 1 h post-mortem ( <i>Semimembranosus</i> muscle)	-0.20 (0.02)		
Lactate (exsanguination blood)	pH 1 h post-mortem ( <i>Semimembranosus</i> muscle)	-0.37 (<0.001)	Slaughter pigs	Rocha <i>et al.</i> , 2015
Lactate (blood sample after lairage)	pH 24 h post-mortem ( <i>Semimembranosus</i> muscle)	0.46 (<0.001)		
Gastrointestinal tract temperature from loading to lairage	CK (exsanguination blood)	0.41 (<0.001)	Slaughter pigs	Sommavilla <i>et al.</i> , 2017
Cortisol (exsanguination blood)	CK (exsanguination blood)	0.45 (<0.001)		
CK (exsanguination blood)	pH 24 h post-mortem ( <i>Longissimus</i> muscle)	0.41 (<0.001)		
Fighting during pre-slaughter mixing	pH 24 h post-mortem ( <i>Adductor</i> muscle)	0.59 (0.01)	Large White and Duroc slaughter pigs	Terlouw and Rybarczyk, 2008
	pH 24 h post-mortem ( <i>Longissimus</i> muscle)	0.33 (<0.05)	Large White pigs	Terlouw <i>et al.</i> , 2005
Heart rate before slaughter	pH 40 min post-mortem ( <i>Longissimus</i> muscle)	0.72 (<0.02)	Duroc pigs slaughtered with low stress	Terlouw and Rybarczyk, 2008
		0.69 (<0.02)	Large White pigs slaughtered with low stress	
Adrenaline (urine, post-mortem)	Meat lightness ( <i>Longissimus</i> muscle)	-0.65 (<0.01)	Nguni cattle	Muchenje <i>et al.</i> , 2009
Noradrenaline (urine, post-mortem)		-0.52 (<0.05)		
Dopamine (urine, post-mortem)		0.53 (<0.05)		
		-0.52 (<0.05)	Bonsmara cattle	
Behavioural score after transportation to the slaughterhouse (calm vs restless)	Meat toughness ( <i>Longissimus</i> muscle; Warner-Bratzler Shear Force)	0.34 (<0.05)	Crossbred steers	Gruber <i>et al.</i> , 2010
Plasma lactate (exsanguination blood)		0.26 (0.002)		

&gt;&gt;&gt;

Table 1.7. Continued.

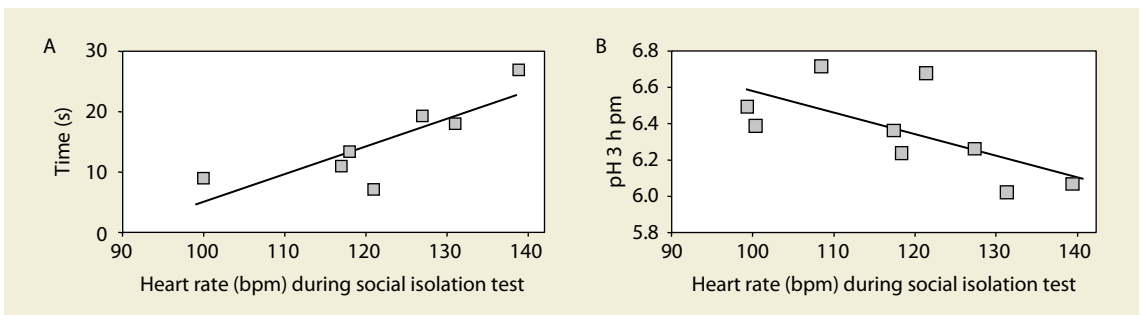
Variable 1	Variable 2	r (p) values	Animal type	Reference
Noradrenaline (urine, post-mortem)	pH 1 h post-mortem ( <i>Longissimus</i> muscle)	0.57 (<0.001)	Young bulls	Reiche <i>et al.</i> , 2019
Heart rate during the first minutes of transport	Time needed to be driven into the abattoir	0.84 (0.003)	Normand cows (supplementary stress slaughter conditions)	Bourguet <i>et al.</i> , 2010
Time needed to be driven into the abattoir	pH 3 h post-mortem ( <i>Semitendinosus</i> muscle)	-0.60 (0.03)	Normand cows (low stress slaughter conditions)	
Heart rate at arrival at the abattoir		-0.64 (0.04)	Normand cows (low stress slaughter conditions)	
Heart rate at arrival at the abattoir	pH 40 min post-mortem ( <i>Semitendinosus</i> muscle)	-0.58 (<0.001)	Young bulls (Angus, Limousin and Blond d'Aquitaine)	Bourguet <i>et al.</i> , 2015
<b>Correlations between reactions during reactivity tests conducted during rearing and meat quality</b>				
N° of contacts with a novel object	pH 24 h post-mortem ( <i>Adductor</i> muscle)	0.83 (0.001)	Large White and Duroc pigs	Terlouw and Rybarczyk, 2008
N° of contacts with an unfamiliar person	Temperature, 5 min post-mortem ( <i>Longissimus</i> muscle)	-0.86 (0.003)	Large White pigs	
Plasma adrenaline levels during restraint	Meat toughness ( <i>Longissimus</i> muscle; Warner-Bratzler Shear Force)	0.22 (0.006)	Crossbred steers	Gruber <i>et al.</i> , 2010
Heart rate response to the presence of the closed umbrella	Meat tenderness ( <i>Longissimus</i> muscle; sensory analysis)	-0.59 (0.01)	Young bulls (Angus, Limousin and Blond d'Aquitaine)	Terlouw <i>et al.</i> , 2021

before slaughter, it may result in a fast early post-mortem pH value decline, while if it occurs during the hours preceding slaughter, it may result in high pH<sub>u</sub> value (Table 1.3). Correlations may also be found for noradrenaline as it is released in concert with adrenaline (Reiche *et al.*, 2019). For example, Foury *et al.* (2011) found that pigs that had been more active (higher plasma CK level at exsanguination) and/or had higher adrenaline or noradrenaline levels had lower preslaughter muscle glycogen content and higher meat pH<sub>u</sub> value (Table 1.7). Muchenje *et al.* (2009) found for cattle of the Nguni breed a negative correlation between adrenaline and noradrenaline levels and lightness of the meat (Table 1.7). In birds, increased physical activity and food deprivation were associated with lower hepatic and muscle glycogen levels (Warriss *et al.*, 1999). Pigs with higher lactate levels after lairage, indicative of greater activity and/or stress levels, had lower pH<sub>u</sub> value in the muscles of the thigh (Rocha *et al.*, 2015; Table 1.7). Muscles that are used for locomotion, during fighting, for mounting other animals or when being mounted, have a greater probability to be influenced by negative slaughter conditions compared to other muscles (Terlouw and Rybarczyk, 2008).

The effects of stress are not limited to the rate and extent of pH value decline and associated meat quality traits, such as colour and water holding capacity. They may also influence sensory traits. In cattle, the use of the electric prod, or back and forwards driving of the animals just before slaughter caused decreases in tenderness and juiciness (Reiche *et al.*, 2019; Warner *et al.*, 2007). The effects are

proportional to the degree of stress as preslaughter behavioural, and physiological stress indicators and beef tenderness showed negative correlations (Gruber *et al.*, 2010; Table 1.7). The relationships between rate and extent of pH value decline and tenderness have been described for pigs, poultry and fish; in beef and lamb, they also exist, but are more complex (Dransfield, 1994; El Rammouz *et al.*, 2004; Huff-Loneragan *et al.*, 2002; Gagaoua *et al.*, 2015; Hwang and Thompson, 2001). It is also important to realise that meat quality is determined not only by metabolic, but also by many other, interdependent, processes (Ouali *et al.*, 2006). For instance, tenderness of the *Longissimus* muscle in cattle was influenced by interconnected pathways involved in muscle contraction, ATP metabolism, muscle structure, oxidative stress and chaperone-mediated protein folding (Gagaoua *et al.*, 2021). Preslaughter stress likely influences all these processes. For example, meat from cows slaughtered following stressful handling presented higher level of oxidation during storage (Delosière *et al.*, 2020).

As stress reactions to the pre- and slaughter context influence meat quality, stress reactivity is a characteristic of the animal that influences its potential for the production of meat and carcasses of high quality. For example, more reactive cattle had higher bruising scores (Fordyce *et al.*, 1988b; Gallo *et al.*, 2022 – Chapter 2 of this book). Pigs that fought more during a food competition test, fought also more when they were mixed with other, unfamiliar pigs during the slaughter period, and therefore had higher ultimate pH and darker meat. The effects on pHu and meat colour were proportional to the level of fighting (Terlouw and Rybarczyk, 2008; Terlouw *et al.*, 2005, 2021; Table 1.7). Normand cows that had faster heart rates when they were removed from their conspecifics during a test conducted three weeks before slaughter, had higher heart rates at the start of transport, where more difficult to drive into the abattoir, and had higher urinary catecholamine levels at slaughter. After slaughter, their *Semimendinosus* muscle, a muscle of the thigh, presented a faster pH value decline and higher temperature, indicative of a faster post-mortem metabolism, compared to their less reactive counterparts (Bourguet *et al.*, 2010; Figure 1.3; Table 1.7). When the closed umbrella was presented to the young bulls described above (Section 1.2.2), their heart rates increased (Bourguet *et al.*, 2015). Following slaughter 3 weeks later, bulls with a greater heart rate increase during presentation of the umbrella had a faster early post-mortem pH value decline in the *Longissimus* muscle (loin muscle). The meat of these bulls was further tougher during sensory



**Figure 1.3.** Correlations between heart rate when the distance between the cow and her group members was increased and (A) time need to introduce her into the abattoir ( $r=0.81$ ;  $P=0.03$ ) and (B) the pH of the *Semimendinosus* muscle 3 h post-mortem ( $r=0.65$ ;  $P=0.06$ ) (Bourguet *et al.*, 2010).



testing (Terlouw *et al.*, 2021; Table 1.7). In the above experiments, the cows and bulls that were more reactive to unfamiliar situations during the tests were probably more stressed by the unfamiliar preslaughter situation, leading to faster ante- and post-mortem muscle metabolism. Other studies showed that a more excitable temperament had negative effects on weight gain during rearing and various meat quality traits, including pH and temperature values decline (Behrends *et al.*, 2009; Café *et al.*, 2011; Gruber *et al.*, 2010; King *et al.*, 2006; Table 1.7). Similar effects were observed in pigs (Terlouw and Rybarczyk, 2008; Table 1.7).

Finally, the influence of preslaughter stress on muscle traits vary according to muscle, breeds, rearing system, feeding regime, physical condition and species amongst others. The effects of stress on different muscles depend on the extent to which the muscles are used in the behavioural response, and their fibre composition, that is, the relative amounts of slow and fast fibres. Compared to slow fibres, fast fibres can be activated rapidly but have fewer mitochondria and therefore, if the demand is high, for a given amount of glucose less ATP is produced. In addition, slow and fast contracting fibres do not have the same glycogen contents. Differences in muscle traits are not necessarily caused by different stress levels. For instance, slow contracting muscle fibres are darker than fast contracting fibres. Certain species, such as pigs and poultry, have more fast contracting fibres and therefore lighter meat than other species, such as cattle and sheep. For the same reason, in pigs, the *Longissimus* muscle has a lighter colour than the *Adductor* muscle, a muscle of the thigh (Terlouw and Rybarczyk, 2008). Thus, the simple observation of lighter coloured meat of an animal cannot be interpreted as an indicator of high preslaughter stress, as other underlying causes are also possible.

## 1.4 Conclusions and outlook

The animal's state of welfare depends on its conscious, subjective experience of emotions. Today, much progress is needed to understand the mechanisms underlying conscious experiences, including in humans. Negative emotions may be indirectly measured, using behavioural and physiological approaches. Many measurements and indicators of stress are relevant for the preslaughter and slaughter period and they can be obtained before or after death of the animal. Physiological variables change due to stress factors of psychological or physical origin, but they are only partly specific. Behavioural indicators are more specific. They need to be combined with observations on the environmental context, and are necessary to interpret the physiological measurements. The combined effects of behavioural and physiological responses influence the characteristics of the meat. Analytical strategies, such as testing reactions to very specific features of the environment, including suddenness, or unfamiliarity, allow a better understanding of which aspects of the environment the animals react to.

Further improvements may be obtained by the development of remote measurements of animal stress and the continuation of the generalisation of internal and external audits. The development of non-invasive tools for the monitoring of animal welfare receives increasingly more attention. Such tools should aim to identify high risk situations. Many different events may be at the origin of high-risk situations; therefore, most efficient would be to monitor the resulting stress state of the animal. Video images can be analysed using algorithms to detect pain or changes in posture and certain behaviours as indicators of illness under experimental conditions or during rearing, but a

standardised recording of such images under abattoir conditions seems still complex (Jorquera-Chavez *et al.*, 2019; Mahmoud *et al.*, 2018). Remotely measured increases in body temperature during slaughter may be a candidate as it is indicative of increased activity and/or stress, as well as a predictor of reduced meat quality (Jorquera-Chavez *et al.*, 2019; Rocha *et al.*, 2019; Weschenfelder *et al.*, 2013). Blood lactate content measured using a hand-held analyser gives similar information, although if used non-invasively on the exsanguination blood, the information is only obtained after the slaughter procedure (Rocha *et al.*, 2015). Increases in heart rate may also be measured remotely, but the animal has to be restrained to be fitted with the system (Jorquera-Chavez *et al.*, 2019). Such tools cannot replace the regular checks carried out by slaughterhouse personnel and veterinarians and should be considered as an additional support. The increasingly frequent use by abattoirs of internal and external audits is further very beneficial for the rapid identification of critical aspects of its organisation and processes and the immediate implementation of corrective actions.

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