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RESEARCHES ON ANIMAL WELFARE AND MEAT QUALITY IN A POULTRY PROCESSING PLANT

Coordinatore
Ch.mo Prof.
Maria Luisa Cortesi

Relatore:
Ch.mo Prof.
Maria Luisa Cortesi

Candidato:
dott.ssa Mariagrazia Girasole

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a Ferdinando

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Abstract

Animal welfare standards are becoming increasingly important as most people feel that animals have the right to a comfortable existence, proper treatment, and humane death.

The European Community in the treaty of Lisbon recognizes animals as sentient beings, capable of feeling pain and pleasure and European Commission has adopted specific programs to improve the animal welfare conditions and to protect them from maltreatment, abuse, pain or suffering during transport, restraint, stunning, slaughter, or killing.

Animal killing for food production and the related operations are events that may induce pain, stress, fear and other forms of suffering to the animals. To face with this problem and guarantee the animal welfare, the EU has adopted the Regulation (EC) N. 1099/2009 on the protection of animals at the time of killing. The Regulation indicates that animals (except some derogation, e.g. the religious slaughter) shall only be killed after stunning, which is necessary to induce a lack of consciousness and sensibility.

The stunning of farm animals prior to slaughtering can be achieved by using mechanical methods (penetrative captive bolt device, non-penetrative captive bolt device, firearm with free projectile, cervical dislocation, percussive blow to the head), electrical methods (head-only electrical stunning, head-to-body electrical stunning, electrical water bath) or gas methods (carbon dioxide at high concentration, carbon dioxide in two phases, carbon dioxide associated with inert gases, inert gases). Electrical water bath stunning is one of the most common methods used to protect poultry welfare in commercial slaughterhouses. Using this

method, the birds are immersed into an electrified water bath, causing the current to run through the head and body, thus inducing unconsciousness.

The present research activity has been carried out in a major commercial poultry slaughter plant. A first aim of the work was to obtain improved animal welfare conditions through the optimization of the stunning procedure. Studies and experimental tests were carried out in order to find an optimal setting of electrical parameters to maximize the number of unconscious birds. Results of both tests and statistical analysis revealed that an increase in the electrical stunning frequency at a chosen stunning current level reduced the stunning effectiveness. So, for a given current level, only a narrow range of electrical frequencies could be employed to achieve an effective stunning.

The maximum probability to have unconscious birds was obtained by using a current level of 200 mA at 400 Hz or 250 mA at 400, 600 and 800 Hz.

The influence of the electrical stunning process on both carcass and meat quality was another object of the present thesis. In particular, the effect of different combinations of electrical stunning parameters (i.e. frequency and current levels) on the occurrence of haemorrhagic lesions has been investigated.

The slaughter plant was also used as an epidemiological lab to investigate on the incidence and nature of myopathies, degenerations and other abnormalities that frequently affect carcasses and meat of broiler chickens. In this context, the incidence of Deep Pectoral Myopathy (DPM or *Oregon* disease) and the incidence of DFD conditions in breast and leg muscles were studied. The effect of factors such as the body weight of the broilers and seasonal factors on the occurrence of these degenerations has been also investigated.

A frequent finding at the cut lab of the slaughter plant was also the occurrence of a myopathy, whose features could not be easily attributed to other muscle degenerations described in literature. The histological characterization of this myopathy and information about its incidence have been reported.

Chapter 1
Animal Welfare and EU Legislation

1.1 Introduction

The safety of the food chain is indirectly affected by the animal welfare, particularly those farmed for food production, due to the close links between welfare, health and food-borne diseases. Stress factors and poor welfare can lead to increased susceptibility to disease among animals. This can pose risks to consumers, for example through common food-borne infections like *Salmonella*, *Campylobacter* and *E.Coli.*

The welfare of food producing animals depends largely on how they are managed by humans. A range of factors can impact on their welfare including housing and bedding, space and crowding, transport conditions, stunning and slaughter methods.

The *transport* of the livestock to the slaughterhouse is one of more stressful events furthermore, the genotype that is selected can influence susceptibility to transport deaths. There is more than one cause for death during the stress that accompanies transport. In poultry it is often linked to congestive heart failure, in pigs to hyperthermia and in sheep to asphyxiation from smothering.

Congestive heart failure is a condition which broiler chickens acquire during the growing period on the farm. The heart is unable to pump blood adequately and blood builds up in the pulmonary veins. This congestion with blood is evident in the lungs, which at autopsy are dark in colour from stagnant blood, and in the swollen pulmonary blood vessels.

Causes of smothering and death from suffocation include poultry panicking during catching and overstocking of poultry transport crates so that weaker birds are trampled and smothered by others. The general signs of asphyxia in the dead animal are relatively non-specific. They can include blood splash, cyanosis and pulmonary oedema, but these can occur in non-asphyxial deaths as well. If an animal takes a long time to die during the smothering, it tries hard to breathe in and this creates sub-atmospheric pressures within the lungs which disrupt the alveolar capillary barrier, leading to the release of a frothy blood into the trachea and mouth.

Mortality from *heat stress* during road transport occurs in chickens and pigs, but it rarely happens in sheep and cattle. A truck may hold 4000 to 5000 closely confined chickens, which together produce considerable amounts of heat. Normally, the birds would be kept cool by wind passing through and between the crates, but if the truck breaks down or gets stuck in a traffic jam, the temperature in the vehicle can quickly rise and some birds will die.

The high risk situations that occur during transport are failure in forced convection (wind), high humidity levels which compromise evaporative cooling from panting, and exposure to solar radiation. During transport the animals are not able to express all the behaviours that normally allow them to keep cool. In poultry, birds seek shade, stretch their wings or legs (to increase surface area and convective heat loss) and pant to increase heat loss.

Dehydration is an added complication in heat-stressed stock on transport vehicles. The effects of severe dehydration include severe thirst, nausea, loss of coordination, and concentrated urine of small volume.

The *pre-slaughter handlings* are important phases for both welfare and meat quality. There are two approaches to trucking animals to meat-works. Small stock can be containerized on the farm and then the container is loaded on to the truck. This approach is used for broiler chickens, end-of-lay hens and most turkeys.

The system for poultry is to load them into crates which are stacked as drawers in a module. The modules are filled with birds by a team of catchers, they drive the birds away from the shed entrance, but it is important, as mentioned before, not to overcrowd the birds otherwise there is a risk of smothering and death from suffocation. The catchers usually grasp the birds by one leg and carry up to seven birds at a time to the module. This procedure can cause damage, especially if the birds become aroused and start wing-flapping whilst they are held. When the birds are being caught the catcher should bend down at the knees to floor level and the birds should be accumulated in one hand with their bodies resting on the floor. Once the correct number has been accumulated they can be lifted. A wrong way is to accumulate birds by one leg in the hand with the birds' other leg standing on the floor. This causes the birds to 'do the splits' and it can lead to hip damage.

When birds are caught and held by the wings, it is important to grasp the wings at the shoulder and not to cause too much pressure on the shoulder joint if it struggles. There is a risk of dislocating the humerus from the shoulder joint in young birds. If the bird actively struggles, pause briefly to try to arrest the movement. Birds should not be held by the neck if it creates a risk of compressing the trachea.

Another difficult situation during pre-slaughter handling is driving the animal up to the stunning position.

In poultry, shackling before electrical stunning is a very stressful operation, as birds are still conscious and have the head upside down. Moreover, the shackles can compress the legs producing distress and pain.

Several studies sustain that when animals are handled quietly at slaughter plants, cortisol levels are similar to those during on-farm handling and restraint (Mitchell et al., 1988; Ewbank *et al*., 1992; Tume and Shaw, 1992; Zavy *et al*., 1992; Grandin, 1993, 1997a).

1.2 European legislation on animal welfare protection

The European Community in the Treaty of Lisbon has recognized animals as sentient beings, capable of feeling pain and pleasure and the Treaty of Amsterdam (EFSA – AHAW/04-027, 2004) has set rules for the actions of the EU in a "Protocol on the Protection and Welfare of Animals". This requires that European Institutions pay full regard to the welfare needs of animals in the formulation and implementation of Community regulations. Animals should be protected from any avoidable excitement, pain or suffering during transport, restraint, stunning, slaughter or killing.

The Council Directive 93/119/EC of 22nd December 1993 stated rules for the protection of animals at the time of slaughter or killing of animals bred and kept for the production of meat, skin fur or other products and indicated methods of killing animals for the purpose of disease control.

For the first time in 2006, the "Community Action Plan on the Protection and Welfare of Animals 2006-2010", adopted by the European Commission, grouped the various aspects of EU policy on animal welfare governing the keeping of billions of animals for economic purposes in the EU. The overall framework for EU action on animal welfare is set out in the EU Animal Welfare Strategy 2012-2015.

In 2009, the EU has adopted the Regulation (EC) N. 1099/2009 on the protection of animals at the time of killing.

In the article 3, the Regulation establishes general requirements for animal killing and related operations and it states that any avoidable pain, distress or suffering shall be spared to animals during these operations. To this aim, the food business operator must ensure that animals are kept protected, clean and in a good thermal conditions. Article 4 states that animals shall only be killed after stunning in accordance with the methods and specific requirements set out in *Annex I*. Moreover, the loss of consciousness and sensibility has to be maintained until the death of the animal. Only in the case of animals subject to particular methods of slaughter prescribed by religious rites, the stunning can be avoided.

Business operators shall also ensure that persons responsible for stunning carry out regular checks to ensure that the stunned animals do not present any signs of consciousness or sensibility in the period between the end of the stunning process and death.

When the outcome of the checks indicates that an animal is not properly stunned, the person in charge of stunning shall immediately take appropriate measures previously set.

Article 17 states that business operators shall designate an animal welfare officer for each slaughterhouse to assist them in ensuring compliance with the rules laid down in the Regulation. It is under the direct authority of the business operator and is in a position to require that the slaughterhouse personnel carry out any remedial actions necessary to meet the Regulation rules.

The responsibilities of the animal welfare officer shall be set out in the standard operating procedures of the slaughterhouse. Also the animal welfare officer shall keep a record of the action taken to improve animal welfare in the slaughterhouse in which he carries out his tasks. This record shall be kept for at least one year and shall be made available to the competent authority upon request.

According to *Annex I* of the Regulation (EC) N. 1099/2009, the stunning of farm animals prior to slaughtering can be performed by using the following methods: mechanical methods (penetrative captive bolt device, non-penetrative captive bolt device, firearm with free projectile, cervical dislocation, percussive blow to the head), electrical methods (head-only electrical stunning, head-to-body electrical stunning, electrical water bath) and gas methods (carbon dioxide at high concentration, carbon dioxide in two phases, carbon dioxide associated with inert gases, inert gases). Stunning must not be carried out unless it is possible to bleed the animals immediately afterwards.

The loss of consciousness and sensibility shall be maintained until the death of the animal. In particular, the duration of unconsciousness induced by a stunning method must be longer than the sum of time that lapses between the end of stun and the time to onset of death.

For poultry electrical water bath stunning, the *Annex I* establishes that the animals shall not be shackled if they are too small for the water bath stunner or if shackling is likely to induce or increase the pain suffered (such as visibly injured animals). In these cases, they shall be killed by an alternative method. The shackles shall be wet before live birds are shackled and exposed to the current. Moreover, birds shall be hung by both legs.

The *Annex I* also indicates the minimum current levels at which birds shall be exposed during water bath stunning (see Table 1), for a minimum duration of four seconds.

Table 1. Electrical Requirements for water bath stunning equipment (average values per animal)

Frequency (Hz)	Chickens	Turkeys	Ducks and Geese	Quails
< 200	100 mA	250 mA	130 mA	45 mA
From 200 to 400	150 mA	400 mA	*Not permitted*	*Not permitted*
From 400 to 1500	200 mA	400 mA	*Not permitted*	*Not permitted*

Chapter 2
Physiological basis for animal welfare

To better understand the animal behaviour during the different slaughter phases, some basic concepts about the physiological responses of animals to external and internal stimuli are provided in this chapter.

2.1 Pain

Broom (2001) described pain as an aversive sensation and feeling associated with actual or potential tissue damage. The neuropsychological system that regulates the perception of pain in man and animals (nociceptive system) has been suggested as an evolutionary protective system.

Differences between man and animals can be found in the cognitive operated reactions to avoid and to cope with a condition of pain (Zimmermann, 2005; Broom, 2001). But also an emotional component of pain is suggested for mammalians as well as poultry (Serviere *et al*., 2009). Though emotional awareness is not necessarily required for nociceptive responses it can be assumed that vertebrates are conscious of pain (Walters, 2008).

Nociception is the general process of encoding and processing of noxious stimuli by the central nervous system. A noxious stimulus is an actually or potentially tissue damaging event. Tissue damage can be caused by a variety of stimuli, including physical, mechanical, chemical and temperature.

The perception of pain is based on an interaction of receptors, nerves, the spinal cord and the brain including the thalamus and the cortex (Brooks and Tracey, 2005).

Pain receptors are located in skin, muscles, joints, periosteum, most internal organs and around blood vessels. Pain can lead to different experiences (e.g. sharp, dull) as different anatomical structures are involved, and different tissue are characterized by different sensors, density of sensors and different types of fibres.

In particular, sharp pain is signalled by A- fibres and the reaction time for perception of sharp pain is short. C-fibres are associated with a slower burning type of pain. Both types of nociceptive fibres innervate the skin and deep somatic or visceral structures (Ringkamp and Meyer, 2008).

During the slaughter process itself pain can be caused by inappropriate restraint, during incorrectly performed stuns and by tissue damage during the neck cut.

There are different types of pain, of which two are welfare relevant during the short time frame of the slaughter process.

Phasic or nociceptive pain results from mechanical or thermal stimuli is also called “brief” or “first pain”.

Tonic or inflammatory type of pain resulting from chemical stimuli released by injury and inflammations is also called “persistent” or “second pain”. During slaughter both forms of pain are produced, nociceptive pain is produced by mechanical forces of cutting and inflammatory pain immediately thereafter by tissue damage. The severity of inflammatory pain can be reduced but not eliminated by a clean cut performed with a sharp knife, while this has little or no influence on nociceptive pain (Brooks and Tracey, 2005).

Pain can be modulated by the central nervous system in both directions (Tracey and Mantyh, 2007).

The nociceptive stimulation of medullary brain centres produces reflex responses including hyperventilation, increased sympathetic tone and catecholamine similar to the stress response, which are further increased by anxiety and fear.

2.1.1 Expression of pain.

Animals can express pain in the following ways (Gregory, 2004):

- ✓ Escape reactions
- ✓ Immobility
- ✓ Abnormal posture, gait or speed, guarding behaviour
- ✓ vocalizing or aggression during movement or manipulation
- ✓ Withdrawal and recoil responses
- ✓ Licking, biting, chewing or scratching
- ✓ Frequent changes in body position – restlessness, rolling, writhing, kicking, tail flicking
- ✓ Vocalizing – groaning, whimpering, crying, squealing, screaming, growling, hissing,
- ✓ barking
- ✓ Impaired breathing pattern, shallow breathing, groaning during breathing, increased rate of breathing
- ✓ Muscle tension, tremor, twitching, spasm, straining
- ✓ Depression, sluggishness, hiding, withdrawal, lying motionless, seeking cover,
- ✓ sleeplessness
- ✓ Avoidance behaviour and aversion to the scene of the trauma
- ✓ Spontaneous autonomic responses – sweating, tachycardia, bradycardia hypertension,
- ✓ vasoconstriction and pallor, increased gastro-intestinal secretions, decreased intestinal

- ✓ motility, increased intestinal sphincter tone, urinary retention
- ✓ Endocrine responses.

The expression of pain differs not only from species to species, but also from individual to individual. Prey species, which live in flocks (e.g. sheep), normally only show very faint signs of pain, as obviously weak or injured animals might attract predators. Individuals within a species vary in the thresholds for the elicitation of pain responses (Gregory, 2004; Broom, 2001).

Recognizing pain can be difficult, because different pain levels or qualities may be expressed differently (Grant, 2004) and some of the signs are not only motivated by pain, like tail wagging and vocalisation (Gregory, 2005b; Grant, 2004; Molony et al., 1995; Molony et al., 1993). During slaughter pain reactions may be masked by restraining device or when the animal is shackled (Holleben, 2009), also the animal may not be able to express a normal response to pain because of the process of slaughter (animals are unable to vocalize if their throat is cut).

2.2 Fear

Fear is an unpleasant emotional condition when anticipating a highly negative event (Sambraus, 1997). Fear and anxiety are two emotional states induced by perception of danger or potential danger, respectively, that threaten the integrity of the animal (Jones *et al*., 2000; Boissy, 1995). Fear and anxiety both involve physiological and behavioural changes that prepare the animal to cope with the danger. Although fear and anxiety have not always been clearly differentiated, fear can be operationally defined as a state of apprehension focusing on isolated and recognizable dangers while anxieties are diffuse states of tension that magnify the illusion of unseen dangers (Rowan, 1988).

General fear becomes a problem particularly when animals encounter new or unexpected stimuli (e.g. a sudden noise or movement, an unfamiliar animal), or situations, e.g. during handling or transportation. This has important implications for animal housing and management. For example, inappropriate handling, corridors/races and pen design, discontinuities in floor texture and colour, drafts and (poor) lighting may all induce fear and its undesirable consequences (Grandin, 2000).

In flock animals collective panic resulting in wild flight impossible to stop can be started by a single animal sometimes provoked by trivial causes like insects (Gregory, 2004).

Fear, anxiety and excitement can heighten the experience of pain via activation the sympathetic autonomous nervous system (Tracey and Mantyh, 2007). Fear and excitement are also important for the effectiveness of stunning methods as they may have an impact on correct positioning of devices and the effectiveness of exsanguination.

2.2.1 Expression of fear

The expression of fear differs widely from species to species and according to individual and genetic differences (Grignard *et al*., 2001; Boissy and Bouissou, 1995; Boivin *et al*., 1994; Grandin, 1993a). Fear in animals can be shown by wide open eyes, freezing reactions or reduced exploratory behaviour, increased frequency of urination and defecation, decreased food intake, longer time before leaving a safe hiding place, increased heart and breathing rate, less salivation, stomach ulcer, increased alertness and agility, licking of the own body and flight intention (Gregory, 2004; Sandem *et al*., 2004a; Sandem *et al*., 2004b; Davis, 1992). Additionally in sheep and cattle the time to approach an unknown object, times without moving, frequency of head rising or delay during feeding can increase (Boissy and Bouissou, 1995; Rushen, 1986).

During the slaughter process a variety of signs of fear can be observed, ranging from obvious restlessness and flight attempts with eyes wide open to simply a paralysed animal with slightly trembling nostrils, which might be licking its lips frequently.

2.3 Distress

Distress is defined in the Guidelines for the Recognition and Assessment of Pain in Animals (UPAW 1989), as a state where the animal has to devote substantial effort or resources to the adaptive response to challenges emanating from the environmental situation. Stimuli potentially leading to distress are thus more or less extreme values or levels of the various factors constituting the animal's environment. Discomfort is looked upon as a mild form of distress. All three terms, pain, distress and suffering are used in European legislative systems. In laboratory animals there are also attempts to classify pain and distress into mild, moderate and substantial (Baumans *et al*., 1994).

2.4 Suffering

Suffering is an unpleasant state of mind that disrupts the quality of life. It is the mental state associated with unpleasant experiences such as pain, malaise, distress, injury and emotional numbness (e.g. extreme boredom). It can develop from a wide range of causes. The European Laboratory Animal Science Associations (FELASA) describes “suffering” as a specific state of 'mind', which is not identical to, but might be a consequence of, pain or distress, which may result in suffering if they are of sufficient intensity or duration, or both. Suffering is reached when pain or distress is no longer tolerable to the individual animal. Physical pain has then reached a level beyond the pain tolerance threshold, or distress has passed the level that the animal is able to cope with. Symptoms of suffering depend highly on the cause of suffering, the individual and the circumstances. Most of the symptoms of pain and fear can also be listed for suffering (Baumans *et al*., 1994).

2.5 Stress

Stress is physiological disturbance, which is closely linked to the mental states mentioned above which is imposed by a stressor, such as a threatening or harmful situation. Stress involves the activation of the hypothalamic-pituitary-adrenal (HPA)-axis and the activation of the sympathetic nervous system (SNS). Activation of the HPA-axis or the sympathoadrenomedullary nervous system leads to increases in heart rate and blood pressure, defecation, suppression of exploratory behaviour, reduced feeding, disruption of reproductive behaviour, exaggerated acoustic startle response, enhanced fright-induced freezing and fighting behaviour and enhanced fear conditioning. The HPA-axis is also activated by trauma and pain (Hellyer et al., 2007; Gregory, 2004).

The SNS is part of the autonomic nervous system which is controlled by certain nuclei in the brain, supplying signals to the sympathetic neurones, which prepare the individual metabolically for the muscular efforts involved in defence and flight. Responses include mobilisation of glycogen and free fatty acids, dilatation of pupils, increased heart rate and contractility and vasoconstriction in those body regions not directly involved in flight or fight mechanisms. Both pathways (HPA and SNS) are interacting, activation of one system can be associated with activation of the other, depending on the stimulus (Gregory, 2004).

2.6 Consciousness and unconsciousness

Consciousness is defined in many different ways, but in general is associated with the awake state and the ability to perceive, interact and communicate with the environment and others

(Zeman, 2001). The opposite state that is, unconsciousness, is defined as: ‘a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence of this disruption, the unconscious animal is unable to respond to normal stimuli, including pain’ (EFSA, 2006). Disruption of brain function can occur as a result of brain concussion, administration of anaesthetics, anoxia or an electroconvulsive shock (Lopes da Silva, 1982). Some authors prefer the term insensibility over unconsciousness, as they find it less anthropomorphic (Blackmore and Delany, 1988).

Insensibility refers to the complete inability to experience any sensations, including unpleasant sensations such as pain (Hemsworth *et al*., 2009).

Stunning of animals aims at inducing unconsciousness and thus insensibility, which lasts until the animal is dead. During the slaughter process, regular checks should be carried out to ensure that the animal does not present any signs of consciousness or sensibility in the period between the end of the stunning process and death (Council Regulation (EC) No 1099/2009, 2009).

Brain regions that are involved in consciousness are the cerebral cortex and thalamus, together forming the thalamocortical complex, which is regulated by the brainstem.

A well-functioning brainstem and thalamus are essential for the maintenance of consciousness and damage to (one of) these regions can cause rapid loss of consciousness (Gregory and Shaw, 2000). However, localised lesions in the cortex, for instance in the sensory cortex, do not necessarily cause unconsciousness, but may only change specific features such as colour vision or the way visual objects and faces are interpreted (Seth *et al*., 2005). The central core of the brainstem is formed by the reticular formation, a large network of neural tissue located in the central region of the brain stem. The reticular formation receives sensory information from the cortex and several subcortical regions and its axons project to the cerebral cortex, thalamus and spinal cord. The reticular formation plays not only a role in sleep and arousal, but also in attention, muscle tone, movement and various vital reflexes (Carlson, 2007). When the reticular formation fails, the cerebral cortex will be switched off or cannot be switched on. When the cortex is (functionally) damaged, neuronal integration of signals from the central nervous system necessary for conscious perception and subjective experience cannot occur. The disruption of normal electrical brain activity is considered to be incompatible with consciousness (Savenije *et al.*, 2002; Lambooij, 2004; Adams and Sheridan, 2008). To maintain consciousness, a constant supply of O_2 and energy to the brain and continuous removal of metabolic waste, such as CO_2, is needed. If one of the mechanisms fails, for instance due to stunning, an animal will become unconscious (Adams and Sheridan, 2008).

2.7 Death

An animal is considered dead when: 'respiration and blood circulation have ceased as the respiratory and circulatory centres in the medulla oblongata are irreversibly inactive. Due to the permanent absence of nutrients and O_2 in the brain, consciousness is irreversibly lost' (EFSA, 2004). In the context of application of stunning and stun/kill methods, the main clinical signs seen are permanent absence of respiration (and also absence of gagging), absence of pulse and absence of corneal and palpebral reflex.

It is important to look at death as a process with different interdependent functions. For example, if the function of the brainstem is sufficiently impaired, respiration will cease. The brainstem is essential for breathing. It is also responsible for the full functionality of the cortex. Thus brainstem death or sufficient damage also leads to the irreversible loss of consciousness. The heart is powered by its own autonomous mechanism. After respiration has ceased the heart will continue to function as long as enough oxygen and energy are available and the waste products can be sufficiently cleared. If cardiac death or sufficient cardiac dysfunction occurs before brain dysfunction, cerebral perfusion will be reduced or stop resulting in the loss of supply of energy and oxygen to neurons within the brain and accumulation of waste products. This causes brain dysfunction and brain death. Correct slaughter will lead to rapid effective blood loss. Consequently energy and oxygen supply progressively falls to the heart and brain and both will stop to function over time (Michiels, 2004).

2.8 Loss of blood volume, loss of blood pressure

The circulating blood volume in animals is estimated to be 8% of body weight and about 18% of total cardiac output flows through the brain at any one time (EFSA, 2004, page 23). With adequate incision of the neck vessels all animals loose between 40 and 60% of their total blood volume and the pattern and rate of loss is similar in the various species examined (Warriss and Wilkins, 1987). Cutting leads to a drop in blood pressure, which may result in hemodynamic instability, interruption of blood supply to the brain and other organs. This can result in insufficient perfusion of tissues with blood, leading to inadequate oxygenation and removal of toxic waste products. Life threatening drops in blood pressure are often associated with a state of shock – a condition in which tissue perfusion is not capable of sustaining aerobic metabolism. The body’s compensatory response to a haemorrhagic shock caused by bleeding, includes systemic reactions such as increased heart rate, local vasoconstriction of arterioles and muscular arteries and shifting of extravascular and venous reserve fluids to the circulating blood volume. This response aims to enhanced cardiac output and maintenance of perfusion pressure, especially in heart, brain and adrenal glands (Guiterrez *et al*., 2008). The time lag between severe haemorrhage and unconsciousness certainly depends on whether and how long compensatory mechanisms are successful or whether they are eventually overwhelmed by blood volume losses (Gregory, 2005a).

The immediate loss of blood pressure after neck cutting has been often described as being important for the rapid loss of consciousness (Rosen, 2004; Levinger, 1995; Levinger, 1976; Levinger, 1961). Mechanisms may be ischemia as well as changes in cerebrospinal fluid pressure (Rosen, 2004; Levinger, 1976; Lieben, 1926).

Irreversible hypovolemic shock and the moribund comatose state result from a loss of more than 50% of the circulating blood volume (Guiterrez *et al.*, 2008). As said above not only the percentage but also the time during which blood volume is lost should be considered.

The results from Levinger indicate that loss of 30% of total blood volume might be reached in cattle at about 60 to 90 seconds after the start of blood flow from the cut neck (a loss of 30 to 40 % of the total blood volume was associated with the loss of consciousness (Gregory, 2005a)). While in sheep this point is reached earlier. Meat chickens slaughtered by religious slaughter lose about 40 % of their total blood volume within 30 seconds of neck cutting (Barnett et al., 2007). The critical values of blood pressure can be reached early in sheep (5 to 6 seconds) (Levinger, 1976). But the blood pressure loss can vary widely between individual animals.

To summarize loss in blood pressure cannot be generally taken as immediate and rapid but variations between species and individual animals exist.

2.9 Cerebral perfusion after neck cutting

The blood supply to the brain of ruminants is derived by a vascular network, the "rete mirabilis occipitale". The rete mirabilis is supplied as well by branches diverging off the carotid artery as by the vertebral artery. It is more extensive in cattle than in sheep. Whereas in goats there are less evident connections between the anastomosis of the two vessels and the rete (Baldwin and Bell, 1963a; Baldwin and Bell, 1963b; Levinger, 1961). Vertebral arteries are also present in poultry (Mead, 2004).

The vertebral arteries of cattle are not severed by the neck cut due to their passage close to the spinal cord. Unlike sheep, the vertebral arteries in cattle are capable of maintaining the cerebral blood flow.

Levinger (1961) concludes from his experiments that the cerebral blood flow through the vertebral arteries would not be sufficient to supply the brain. Nevertheless, even if the blood flow from the vertebral arteries may not be sufficient to supply the whole brain, it is likely that it contributes to prolong brain function and consciousness.

In some animals vertebral artery blood flow increased substantially following sticking.

Daly *et al.* (1988) suggested two explanations: first there are differences between animals in the proportion of the total cerebral blood flow which is contributed by the vertebral arteries. Secondly the amount of blood reaching the brain via the vertebral arteries after slaughter is very close to the minimum blood flow necessary to sustain electrical activity in the brain cortex, so that slight differences in individuals would result in large variations (Daly *et al.*, 1988).

Cerebral hemodynamic compensatory mechanisms will also help to maintain brain function during reduced systemic blood pressure. Cerebral perfusion pressure (CPP), the driving force for blood through the cerebral circulation is defined as the difference between mean arterial pressure and venous backpressure or intracranial pressure. As CPP falls, cerebral blood flow is initially maintained by vasodilation of resistance arterioles, a reflex known as autoregulation. With further reductions in CPP, the autoregulatory capacity is exhausted and cerebral blood flow falls as a function of pressure, but increases in oxygen extraction fraction will maintain cerebral oxygen metabolism and tissue function up to a point (Derdeyn, 2001).

Therefore, the factors influencing the dynamics of cerebral blood flow after neck cutting seem to be very complex and individual differences as well as age, weight and breed have an impact so that the picture given by the above named investigations on cerebral blood perfusion is still incomplete with regard to explaining prolonged consciousness after the cut.

2.10 Impacts on bleed-out or exsanguination

Gregory (2005b) gives an overview on the factors affecting bleeding, which are further explained below:

- Blood vessels that are severed;
- State and patency of the sticking wound;
- Cardiac arrest at stunning;
- Orientation of the carcass;
- Vasodilatation and vasoconstriction in the capillary bed;
- Tonic muscle contraction squeezing blood capillaries and vessels and
- Clonic activity causing movements of blood towards the sticking wound.

In sheep, bleeding out by cutting both the common carotid arteries and the jugular veins is the quickest method of abolishing brain responsiveness (loss of visual evoked responses, relevant EEG changes) compared to cutting only one carotid artery, only the jugular veins or cardiac ventricular fibrillation (Gregory and Wotton, 1984a; Newhook and Blackmore, 1982b).

In poultry cutting both carotid arteries, compared to cutting one common carotid artery and/or one jugular vein, induces impaired brain function most rapidly (Gregory and Wotton, 1986).

Following the cut the severed ends may retract below the wound surface, so that they are covered by surrounding muscle tissue. Because carotids and trachea are linked by connective tissue, respiratory movements can cause the backward movement of the trachea within the thoracic cavity, this may further cause disruption of blood loss from the carotids. Certain positions of the animal during bleeding may facilitate this effect (Levinger, 1995; Anil *et al.*, 1995a; Hoffmann, 1900). Rosen (2004) mentioned the importance of correct post cut restraint with regard to correct bleed-out and time to loss of consciousness. It has been suggested in cattle that when inverted bleeding might be impaired. This is suggested has been the result of the weight of the abdominal organs pressing on the diaphragm and major veins. The added pressure on the heart may decrease stroke volume (compare "cardiac tamponade") and the pressure on the veins may impair venous reflux (Adams and Sheridan, 2008).

The effects of animal's position on bleeding rate may have been previously overestimated, e.g. in sheep bleeding is slightly more rapid in a recumbent position than if suspended in a vertical position (Blackmore and Delany, 1988). In cattle Hess (1968) after captive bolt stunning, recovered more blood from a hanging carcass than in a recumbent position. Another investigation comparing recumbent and hanging position after electrical stunning and hanging position after captive bolt stunning produced similar results in all methods. It has been concluded, that the capability of the person performing the cut is more important than stunning method or position of the carcass (Bucher et al., 2003).

It is possible that differences between brain size, blood volume, and arterial cross sectional area, especially with increasing body size may have an effect on the time to loss of consciousness. The carotid arteries of adult cattle may be too small relative to total blood volume to allow for sufficiently fast bleed-outs and a drastic loss in blood pressure. It is further suggested that in sheep and cattle different percentages of the total blood volume are necessary to supply the brain (Adams and Sheridan, 2008).

This argument may be supported by the fact, that due to practical aspects many studies on time to loss of consciousness have been conducted on smaller animals, e.g. sheep and calves and that these results differ from most findings under practical conditions for full grown animals.

Finally there is also a possible role of the sympathetic nervous system, e.g. if this is activated by preslaughter stressors leading to changes in regional blood flow and slow bleeding rate. Catecholamine release by preslaughter stressors can affect the distribution of blood between the peripheral vascular beds, from where blood is shifted into the central large vessels in case of stress (Warriss and Wilkins, 1987) and consequently more blood loss is required to achieve unconsciousness. In this context the severance of the vagus nerve has to be discussed (Gregory, 2005b). Gibson et al. (2009a) found, that the drop of blood pressure following transection of the ventral neck tissue without disruption of blood circulation was immediate and more pronounced than after blood vessel transection without severing the neck tissue, which was however similar to slaughter by ventral neck cutting of intact animals (Gibson et al., 2009b; Anil et al., 2006). Gibson et al. (2009a) assumed that the effect on blood pressure by cutting the neck tissue without cutting the major blood vessels was due to the severance of the vagosympathetic trunk. To summarize there are manifold impacts on the quality of bleeding and thus the time to loss of consciousness, some of which cannot be mitigated by the performance of the cut.

2.11 Principles of restraint and requirements for restraint

Restraining means restricting the movement of an animal/holding the animal in a correct position, so that a procedure (e.g. sticking or stunning) can be carried out accurately. The ideal restraining method for slaughter depends on the animals to be slaughtered, the method of slaughter (including slaughter speed and the process for stunning and/or cutting) and the capabilities of the staff. There are some basic principles of restraint with regard to animal welfare which have to be fulfilled independently from the slaughter method (Holleben, 2007):

- An animal should be able to enter / to be put in the restraining device without stress;
- restraining itself must cause as little stress / strain as possible;
- restraint time should be as short as possible;
- restraining must not cause injuries;
- when a mechanical or electrical stunning method is applied the restraining method must allow the secure positioning of stunning devices; when slaughter is performed without stunning the restraining method must allow the correct application of the bleeding cut;
- prompt back up stunning / stunning in case of prolonged consciousness or recovery must be possible;
- if bleeding is not carried out in the device a quick release of the animal must be possible to guarantee a short stun-stick interval;
- a restraining device or method must suit the size and species and type of animals slaughtered;
- restraining must not cause negative impact on bleed-out, carcass or meat quality and should match the intended slaughter speed;

- good working safety must be achieved.

Animals enter a restraining device more easily, if there are no impediments like air draughts, sudden hissing or banging noises, dark areas, sparkling reflections, moving people or parts of the slaughter chain, slippery floor, inadequate floor incline or changes of structure or colour of walls or floor, and if the restraining device is well designed, e.g. shield the animal from distractions or does not appear too much a dead end.

Consequently the stress and strain an animal experiences during restraint depends on quality of raceways towards the restraining device, construction of the restraining system itself, the degree of restraint (tightness or pressure), the time of restraint and individual experience e.g. during pre-slaughter handling or individual features of the animal (excitement, adverse reactions, weight, horns) (Grandin, 1998b; Grandin, 1996; Grandin, 1994b).

The restraining method should not cause defence movements or flight reactions of the animal, which can lead to incorrect procedures due to wrong positioning of the stunning or cutting instruments (Holleben, 2007). All restraining methods should use the concept of optimal pressure. The device must hold the animal firmly enough to facilitate slaughter without struggle but excessive pressure that would cause discomfort should be avoided. Struggling is often a sign of excessive pressure (Grandin, 2005).

Smaller animals can be lifted into the restraining device by hand, e.g. sheep and goats may be put by hand on a table or poultry are put in shackles or funnels. These animals may be also restrained by hand without the help of a sophisticated mechanical device. Heavier animals like cattle need more complicated technical equipment as well to hold them, e.g. if they break down, but also to ensure working safety (Holleben, 2007).

Knowledge and skills of the staff handling the animals and operating the devices is extremely important for reducing stress, strain and injuries during fixation and restraint and also for eliminating negative impacts on bleed-out, carcass and meat quality (Grandin, 1998a).

With electrical and mechanical stunning methods it is important to place the stunning device accurately on the head. This usually requires individual restraint of the animals.

Bleeding is performed either in the restraining device or on the shackled animal after it has been released from the restrainer.

An inadequate restraint can lead to incomplete stunning by misplacement or interrupted application of the stunning device such as tongs and captive bolt gun. It can also lead to late bleeding if the animals are not transferred sufficiently quickly to the bleeding position (Adams and Sheridan, 2008; EFSA, 2004). In cattle concave shaped tables for the head in combination with a back pusher improve targeting bolt position in cases of high slaughter speeds (Holleben, 2007). However too tight fixation of the head (e.g. by a chin lift and neck yoke in a poorly designed system) will lead to increased stress and prolonged times until head restraint (Ewbank, 1992).

Sheep naturally follow each other and will often line up and freely enter a well-shaped restrainer or trap, usually showing little or no agitation. However, incompetent handling, such as grabbing of fleece or putting pressure on wrong parts of the body during manual restraint will lead to increased stress and arousal (Hutson, 1993). When sheep are group stunned in a pen, they may hide their heads under animals which makes it difficult to correctly place the electrodes. Also other sheep in the group may make physical contact with the animal that is being electrically stunned possibly resulting in painful electrical shocks. Sheep should be individually restrained manually in a trap or in a restrainer, to minimise incomplete stunning and painful electric shock from group mates (EFSA, 2004). In poultry most concerns have been expressed on the practice of live bird shackling. The pressure applied during shackling increases with deformation of legs or increasing weight and size especially in turkeys. Nevertheless, some modern shackle lines are designed to accommodate birds of different sizes but these are not commonly used under the existing processing conditions (Gentle and Tilston, 2000; Gregory, 1998b; Gregory and Wilkins, 1990; Gregory *et al*., 1989). Shackling time has been limited in the respective European slaughter legislation and even the phasing out of live birds shackling is being discussed. In gas stunning systems poultry may either stay in their transport crates or they may be tipped automatically from the crates onto a belt conveyor. Consequently there is no need for individual restraint in these systems. Dump module systems used for tipping the birds out of the transport crates must be constructed in a way to achieve the birds sliding – not falling – out of the crates onto a sufficient large area of the belts. This is necessary to minimize the higher frequency of red wingtips from wing flapping, associated with some of these systems.

Chapter 3
Stunning methods in livestock

Effective stunning removes the risk that animals will experience pain and distress during slaughter and subsequent bleeding. Additionally, in a stunned animal the cut will be easier to perform. This helps to sever the blood vessels more accurately and achieve a rapid bleed-out especially in large livestock (Gregory, 1998c).

Since the intention of a “humane” slaughter is to avoid or minimize anxiety, pain, distress or suffering at slaughter, stunning methods should ideally induce immediate and unequivocal loss of consciousness and sensibility. Efficient stunning methods, in fact, disrupt the neurons and neurotransmitter regulatory mechanisms in the brain. When loss of consciousness is not immediate, the induction of unconsciousness should be non-aversive. As also indicated in the Regulation (CE) N. 1099/2009, the duration of unconsciousness induced by a stunning method should be appreciably longer than the sum of the time interval between stunning and sticking plus the time it takes for blood loss to cause death. Sticking should therefore be performed quickly after the stun and, in this process, the major blood vessels supplying oxygenated blood to the brain must be severed to ensure rapid onset of death (EFSA, 2004). Moreover, animals showing signs of return of consciousness must be re-stunned immediately using an appropriate back-up method.

3.1 Electrical stunning

Electrical stunning induces unconsciousness and insensibility by producing a depolarization shift in nerve cells followed by hyperpolarisation of action potentials which leads to epileptiform discharges (Gregory, 1987b). If an electric current is applied to the head, and sufficient current flows through the brain, unconsciousness occurs in a similar manner to that produced in grand mal epileptic seizure. Grand mal epilepsy is a pathological extreme of neuronal synchrony and is considered to be incompatible with normal neuronal function and, hence, persistence of consciousness (Cook et al., 1995; Cook et al., 1992).

After that the electrical current flows through the brain there is a dramatic rise in the extracellular concentrations of Glutamate and Aspartate (excitatory neurotransmitters). The cell structures are in a state of heightened excitation and uncoordinated activity. The interruption in the processing of signals leads to an immediate loss of consciousness (within 200 ms (Cook et al., 1995)). The slower release of GABA (inhibitory neurotransmitter), then, brings the seizure to an end. Because elevated concentrations of GABA can also be due to stress and elevated GABA levels can inhibit the effects of Glutamate/Aspartate, stress can negatively influence the ability to produce epilepsy. This also illustrates the importance of careful handling of animals prior to stunning. The elevation of the extracellular concentration of GABA lasts significantly longer than that of Glutamate/Aspartate and contributes to a long lasting analgesia after the electrical stun (5-15 min).

Two kinds of electrical methods are used at present (EFSA, 2004):

- *Electrical head only stunning*: involves transcranial application of an electric current in red meat species and poultry, in the latter however the current can also be applied through the whole body (*water bath*).

- *Electrical head to body stunning*: usually involves head-to-body application of an electrical current in red meat species and sometimes in poultry.

Depending on electrical frequency used, these methods can produce ventricular fibrillation in the heart and thus lead to a prompt and sustained decrease in blood pressure and avoid resumption of consciousness if bleeding is too late or badly performed (Gregory and Wotton, 1984b).

The occurrence of ventricular fibrillation depends on:

- the pathway the current takes through the body;
- the region of the heart that receives the current;
- the phase of the heartbeat cycle which coincidences with the start of the current;
- the duration of current flow;
- the frequency and waveform of the electrical current (high current frequencies are less likely to induce a ventricular fibrillation)(Gregory *et al*., 1991);
- the species, as the heart in species with high intrinsic heart rates is less readily fibrillated with electrical currents (Gregory, 1998a).

Thus by influencing the current parameters and pathway, ventricular fibrillation can be avoided. As ventricular fibrillation or cardiac arrest present a risk of being painful to the animals, it is essential to stun the animal before or at the same time as inducing cardiac arrest (Gregory, 1998a). When an animal is electrically stunned by head-only stunning there is a prompt fall in heart rate whilst the current is flowing, but when the stunning current is switched off heart rate rapidly rises to above normal rates (Gregory, 1998a). During the current flow through the brain, the body of the animal becomes rigid, because brain stimulation and electrical impulses passing down the spinal cord cause tonic muscle contraction. The hind legs are flexed and if its weight is not mechanically supported, the animal would fall to the ground. When the current flow stops, the generalized tonic contraction usually continues for a short period (tonic phase, e.g. 10 seconds) and then convulsions (clonic phase) set in. These convulsions are driven by dysfunction of certain brain structures, e.g. reticular formation (Gregory, 1998a). The return of rhythmic breathing indicates that hypersynchrony of brain neurons has ended and some of the normal function has been restarted. Other functions will probably follow, and resumption of consciousness is impending. If there is no or insufficient bleeding after electrical stunning, without induction of ventricular fibrillation, animals will start rhythmic breathing and will soon regain consciousness (Gregory, 1998a).

The signs of a successful electrical stun are: immediate collapse of free-standing animals (not be applicable to poultry restrained in a cone or shackle or animals held in a restraining conveyor); immediate onset of tonic seizure (tetanus) lasting several seconds, followed by clonic seizure (kicking or uncoordinated paddling leg movements), applies to all red meat species but not always to poultry electrical stunning; apnoea (absence of breathing) lasting throughout tonic-clonic periods; upward rotation of eyes (except for poultry).

In cattle and calves the major challenges with head-only electrical stunning are the short duration of the epileptiform insult and the occurrence of strong clonic convulsions. Various studies have shown that the duration of unconsciousness, measured from the resumption of normal breathing, is between 20 and 90 seconds.

Scientific evidence resulting from analysis of electroencephalogram (EEG) and neurotransmitters indicates that correct head only electrical stunning followed by neck cutting within 10 seconds is an effective method. Both, stunning and neck cutting additively increase the respective neurotransmitters in the brain, which implies that electrical stunning accelerates brain failure after sticking due to its exhaustive effect on brain metabolism (Cook and Devine, 2003). The recommended minimum amperage is 1.28 amperes for adult cattle and 1.25 amperes for calves up to 6 month of age (*Annex I* of Regulation (CE) N.1099/2009)

Electrode position for handheld tongs is preferably temporal between the eye and the ear. With automatic current application the current flows through the brain between neck electrodes and a nose plate. Current should be applied for at least 4 seconds to the head. Under routine conditions, effectiveness of electrical stunning however may be low, due to technical shortcomings.

In sheep and goats the main principles apply as for cattle. The tongs should be positioned between the eyes and the base of the ears on both sides of the head preferably on local wet skin. Wool, dry skin and placement of the tongs in a caudal position behind the ears, lowers stunning effectiveness (Velarde *et al*., 2000). Effective head-only stunning in sheep should be induced using minimum currents of 1.0 A (*Annex I* of Regulation (CE) N.1099/2009). A minimum of 250 V should be used to deliver the current. Duration of current flow should be a minimum of 2 seconds. Following anecdotal reports for mature sheep even higher intensities of current about 1.3 to 1.5 A may be necessary to achieve sufficient stunning effectiveness. Currents used in sheep in practice often have a higher frequency than 50 Hertz (e.g. 100 or 400 Hertz) and also current patterns are used where the frequency decreases during current application from 500 Hertz to 100 Hertz. In order to check for clinical signs of correct stunning and recovery in sheep, the safest indicators are the typical pattern of seizures and return of normal rhythmic breathing. Resumption of rhythmic breathing can occur during the second clonic phase, as in lambs the seizure activity after high voltage head-only stunning includes a tonic and two clonic phases (Velarde *et al*., 2002).

In poultry two electrical stunning methods are currently used: *head-only electrical stunning*, where the current is applied only to the head via a pair of electrodes, and *electrical water* *bath stunning* where birds are immersed, generally up to their shoulder, into an electrified water bath. For both methods the depth and duration of unconsciousness depends upon the amount and frequency of currents applied (EFSA, 2004)

In conclusion, electrical stunning is a "humane" method to have an animal instantaneously unconscious and with timely and effective bleeding unconsciousness and insensibility will last until death supervenes by bleeding. Nevertheless, it must be guaranteed that the necessary technical requirements are fulfilled under routine conditions, as welfare can be poor in case of noncompliance.

3.2 Mechanical stunning

Among mechanical stunning methods the *penetrating captive bolt* method, has to be distinguished from the *non-penetrating* method. The *non-penetrating method* is sometimes called *"concussive stunning"*, though concussion is the underlying principle for both methods. Both types of gun are normally fired on the forehead (usually frontal bone) of an animal, but other sites may be selected when there are horns or thick ridges on the skulls. Captive bolts must always be fired perpendicular (at right angle) to the skull bone surface (at the chosen site); otherwise bolts may skid and fail to fully impact the skull.

3.2.1 Penetrating captive bolt stunning

During penetrating captive bolt stunning there is structural damage to the brain in addition to the concussive impact on the skull. Using this method, a steel bolt is powered by cartridges or compressed air, and for poultry the bolt may be spring driven. The bolt is not pointed, but the tip is sharpened in a concave manner and has a sharp rim without nicks. To achieve good stunning the captive bolt device must be correctly placed and a bolt of adequate length and diameter must be sufficiently accelerated. Consequently there will be transfer of energy to the animals head, causing concussion and structural damage as the bolt travels through the brain. Immediate insensibility and unconsciousness is caused by rapid propagation of shockwaves of kinetic energy through the brain and abrupt acceleration and deceleration of the relatively soft brain within the bony skull (shear and contre-coup effects). This impact can be short lasting. For different species and sizes of animals, different guns are used, these have differing mass, length and diameter of bolts. Specific cartridge strengths or air operating pressure are also used for different animal types and there are specific shooting positions on the animals head for different species. If the bolt is too thin or it is fired through a trephined skull there will not be enough energy transfer to the head to induce effective stunning (Raj and O'Callaghan, 2001).

Effective stunning can be monitored from immediate collapse and prompt, persistent absence of rhythmic breathing. The muscles in the back and legs go into spasm, fore legs and hind legs are flexed, the fore legs straightening after a few seconds. Signs that indicate a shallow depth or concussion include flaccid muscles immediately after stunning, return of rhythmic breathing and rotated eyeballs. Return of rhythmic breathing happens if stunning is insufficient or bleeding is too late (Gregory, 1998c).

Heart activity after captive bolt shooting in cattle can continue for about 4 minutes in animals that are bled immediately following stunning, but can continued for 10 minutes in animals that are not bled (Vimini *et al*., 1983). Schulze *et al.* (1978) also found persisting activity of the heart and described an increase of heart rate up to 300 beats per minute after stunning in sheep and calves. According to Kaegi (1988) heart rate and blood pressure increased after captive bolt stunning of cows due to activation of the respective brain centres. Captive bolt guns can be either trigger or contact fired. With contact fired guns, there is no possibility to correct the position of the gun once it touches the head of the animal. As a consequence there are more failed shots with this type of gun in some plants depending on the skill of the staff. Bad maintenance of guns is often the reason for stunning failures. Deformed bolts will not achieve the necessary speed, also too short or narrow bolts can lead to decreased stunning effectiveness, through decreased kinetic energy transfer (Gregory, 2007; EFSA, 2004).

Effective captive bolt stunning is associated with immediate absence of evoked cortical responses in the brain (Daly and Whittington, 1989; Daly *et al*., 1987). Absence of primary cortical evoked responses indicates failure in neurotransmission at a level that occurs before conscious perception of a stimulus. Unlike evoked responses, the spontaneous EEG is not as reliable as an indicator of brain disturbance following captive bolt stunning (Gregory, 2007; Daly *et al.*, 1988; Daly, 1987).

In *cattle*, to ensure efficient stunning, the captive bolt must be fired at the crossover point of imaginary lines drawn between the base of the horns and the contralateral eyes and certainly no further away than 2 centimetres radius from this point (EFSA, 2004, Lambooij *et al*., 1983; Lambooij, 1981a; Lambooij, 1981b). Kaegi (1988) gives the outer corner of the eye as reference point to the base of the horns, thus slightly moving the aim upwards. Shooting accuracy becomes more critical using low powered devices (Gregory, 2007). Deviations from the recommended shooting position and from the perpendicular shooting direction increases intensity of muscle spasms after shooting and this may impede further processing including hoisting and sticking (Marzin *et al*., 2008; Kaegi, 1988).

Close restraint of the animal’s head is generally necessary for exact aiming with this stunning method, because otherwise the operator cannot follow the animal’s head movements and revise the aim. Bolt velocities have to be above 55 m/s for steers heifers and cows and 70 m/s for young bulls that are usually more difficult to stun. The transfer of energy to the head and the depth of the stun are improved when bolt diameter is 16 mm or more (Gregory, 2007). To conclude, captive bolt stunning, when fired with appropriate devices using correct cartridges or air pressure and applied accurately, induces reliably effective stunning in all adult cattle and calves. It is nevertheless very important with this method to reliably re-stun animals when poor stunning is suspected. Bleeding within 60 seconds can pre-empt suffering in cases of slight deviation of optimum performance.

In *sheep* and *goats* generally the same principles apply as for cattle. Ideal shooting position for polled sheep is the highest point of the head in the mid-line, pointing straight down to the throat. The ideal shooting position for horned sheep and for all goats is the position just behind the middle of the ridge that runs between the horns. Then the captive bolt should be aimed towards the mouth (EFSA, 2004). Changing the shooting position from the frontal position to the poll may alter the mechanics of the impact such that the diffuse damage to the brain is reduced, possibly owing to reduced acceleration of the head and can be associated with rapid recovery of brain function in sheep (average: 50 s, earliest: 33 s after the shot). Therefore, shooting in the poll position should only be used when it is essential (i.e. in horned animals) and then always followed promptly by sticking within 16 seconds (Daly and Whittington, 1986). Based on practical experience, behaviour post-stunning is very similar to that seen in cattle, indeed there is prompt and persistent apnoea and immediate onset of tonic seizure, the position of the eyeball is fixed, i.e. facing straight ahead (EFSA, 2004). During bleeding strong clonic convulsions can occur.

Failed stunning can occur due to wrong positioning of the stunner. However when performed correctly, penetrative captive-bolt stunning is an effective method of stunning sheep and goats, and loss of consciousness is immediate.

3.2.2 Non-penetrative captive bolt stunning (concussive stunning)

Depending on the amount of brain damage induced*,* non-penetrative captive bolt stunning can cause either permanent or temporary unconsciousness. In a study on *lambs* and *calves*, the majority showed signs of recovery (Blackmore and Delany, 1988, page 57). These signs and the development of righting reflexes did not usually occur in less than 2 minutes (Blackmore, 1979). To ensure effective stunning in adult *cattle*, the non-penetrative captive bolt must be placed 2 centimetres above the cross-over point of imaginary lines drawn between the base of the horns and the contralateral eyes. This must be achieved very precisely using proper body and head restraint, because only slight variation in the ideal shooting position and angle decreases stunning efficiency (Grandin, 2003; Hoffmann, 2003). Endres (2005) suggests that massive hair on the forehead or moulding of foreheads hinders good contact of the concussive head to the bone and thus lead to decreased energy transfer. Studies on effectiveness of the method reveal different results. Finnie (1995) after studies on 12 adult cattle found that frontal non-penetrative captive-bolt-stunning resulted in immediate loss of consciousness in all animals, as indicated by immediate collapse and absence or rhythmic breathing. Whereas Lambooij *et al.* (1981) using electroencephalographic methods could only produce immediate unconsciousness in 15 out of 19 veal calves of 200 kilograms live weight. Gibson *et al.* (2009d) tested the electroencephalographic and cardiovascular responses of halothane-anaesthetised calves (109 to 144 kg live weight) to non-penetrative captive-bolt stunning and showed that non-penetrative captive-bolt stunning virtually instantaneously altered cerebrocortical activity. Immediately after stunning, respiration ceased in all calves. Some animals exhibited slow uncoordinated limb movements during the first 5 seconds. The frontal bone of all calves had a 30-millimeters diameter circular depressed fracture at the site of impact of the bolt, with adjacent subarachnoid haemorrhage and physical damage to brain tissue. Diffuse damage was also seen throughout the brain, manifested as traumatic axon injury, brain swelling and haemorrhage (Gibson et al., 2009d).

Fracturing of the skull results in less effective stunning (Endres, 2005). Effective re-stunning can only be achieved by penetrating captive bolt, as using a second non-penetrative blow did not transfer enough energy to the brain because of swelling and fracturing (Endres, 2005). In cattle, sticking should be performed within 12 seconds of non-penetrative captive bolt stunning (EFSA, 2004), and if possible even earlier (Mintzlaff and Lay, 2004). Heart activity continues after slaughter as with penetrative captive bolt stunning in adult cattle and veal calves (Gibson et al., 2009d; Hoffmann, 2003; Lambooij et al., 1981). In calves, outward signs of effective non-penetrative captive bolt stunning were described as the appearance of 5 to 15 seconds of tonic convulsions and spasms prior to relaxation, or as extensor rigidity and some generalised muscular tremors, followed by slow hind leg movements. Absence of rhythmic breathing lasted for up to 35 seconds, and absence of righting behaviour lasted for a minimum of 60 seconds (Lambooij *et al*., 1981; Blackmore, 1979).

When using a non-penetrative captive bolt in *sheep* as well as cattle, unconsciousness should be induced with a single blow at the frontal position of the head. Subsequent shots may not be effective due to the swelling of the skin occurring from the first shot, and therefore, should not be allowed. If the first shot is unsuccessful, the animal should be stunned immediately using a penetrating captive bolt or electric current (EFSA, 2004). Therefore, non-penetrative concussive stunning in cattle and sheep is not satisfactory so far from the animal welfare point of view, due to the relative high failure rate. Improvements seem to be possible by developing the shape of the bolt, better fixation of the head, and standardisation of cartridge power as well as shape of the bolt in relation to different age groups and genetic lines (Moje, 2003).

3.3 Gas stunning

Under commercial conditions, gas stunning methods are mainly used in pigs and poultry.

In *poultry*, the main advantage of gas stunning is that the birds can be stunned in groups. In this way, handling is reduced and shackling of conscious poultry and any associated negative effects on poultry welfare are avoided. Use of gas stunning in poultry will be described in detail in other parts of this thesis.

In *pigs*, CO_2-stunning systems have increased in popularity due to their positive effects on meat quality compared, for example, with electrical stunning (Velarde et al 2000, 2001). In commercial conditions, animals are immersed into a concentration gradient of the gas, such that, as the cage is lowered into the well, the CO_2 concentration continues to rise until it reaches 80–90% at the bottom of the well.

The inhalation of high concentrations of CO_2 induces hypercapnic hypoxia in the animal and leads to changes in blood parameters, such as pH, carbon dioxide partial pressure (pCO_2), oxygen partial pressure (pO_2), oxygen saturation ($SatO_2$) and bicarbonate concentration (HCO_3^-) (Lomholt 1998; Martoft *et al*, 2001). Consequently, there is a decrease in the pH of cerebrospinal fluid (CSF) and the animal loses consciousness (Gregory, 1987). However, acceptability of CO_2 stunning on welfare grounds has been questioned. This because loss of consciousness is not immediate (Raj & Gregory 1995), and is dependent upon the CO_2 concentration and the speed at which animals are immersed into the greatest concentration of gas at the base of the well (Troeger & Woltersdorf 1991; Raj & Gregory 1996). Signs of aversion, such as retreating and attempting to escape have been described in pigs during the inhalation of CO_2 at concentration above 30% (Raj & Gregory 1996; Velarde *et al*, 2007). Raj and Gregory (1995) reported that pigs tried to escape from an atmosphere of 90% CO_2

in less than 5 s. Troeger and Woltersdorf (1991) observed that immediately after immersing pigs into the CO_2 gas mixture (60 to 90%), they backed away and sniffed. The effects of CO_2 are two-fold; firstly, it causes irritation of nasal mucosal membranes and lungs (Peppel & Anton 1993), where the presence of chemoceptors acutely sensitive to this gas has been described (Gregory *et al*, 1990) and, secondly, it is a strong respiratory stimulator that provokes hyperventilation (Gregory *et al*, 1987), and a sense of breathlessness prior to loss of consciousness (EFSA 2004).

During inhalation, loss of posture has been considered the first behavioural indicator of the onset of unconsciousness (Raj and Gregory 1996). At this point, pigs demonstrate muscular excitation. Forslid (1987) found that low frequency activity in the EEG (> 4 Hz) became the dominant signal prior to the start of convulsions and suggested that pigs were unconscious before this muscular excitation phase. Conversely, Hoenderken (1983), who also analysed changes in the EEG, stated that unconsciousness appeared after the muscular excitation period and, as a result, these body movements were voluntary escape attempts. This lack of agreement is due to difficulties determining the exact moment at which the loss of consciousness occurs from the amplitude and frequency of the EEG.

Auditory evoked potentials (AEP) have been suggested as being a more precise indicator of the level of consciousness than the EEG (Thornton et al 1989).

Under commercial conditions, the absence of a corneal reflex has been used to assess the state of unconsciousness in pigs after CO_2 stunning,

Chapter 4
Stunning of poultry

4.1 Introduction

A basic requirement for "humane slaughter" of animals including poultry is that they should be stunned, i.e. rendered unconscious and insensible, prior to slaughter by exsanguination. As also described in the previous chapter, under commercial conditions, red meat animals (horses, ruminants and pigs) are generally stunned using either a penetrating captive bolt, non-penetrating concussion bolt, electric current or carbon dioxide gas, whereas poultry species are often stunned by using an electrified water bath. There are two main reasons for this common practice in poultry. Firstly, it may be difficult to apply captive or concussion bolt without compromising bird welfare under commercial conditions due to a very small target area (head) for shooting in poultry. Secondly, the throughput rates required in medium to large chicken processing plants (up to 9 thousand birds per hour are killed) can be easily achieved with water bath stunners rather than with captive bolt or concussion stunners. An alternative stunning system for broiler chickens, which is becoming more and more used in recent years, is gas stunning (CAS = Controlled Atmosphere Stunning). This stunning method allows to eliminate the stresses associated with uncrating and/or shackling of live birds prior to electrical stunning.

Stunning of slaughter animals is applied to induce a state of unconsciousness and insensibility of sufficient duration to ensure that the animal does not recover while bleeding to death (exsanguination). Moreover, stunning should produce sufficient immobility to facilitate the initiation of exsanguination. It is generally stated that unconsciousness and insensibility should be induced as soon as possible and without a detrimental effect on the welfare of the animals and the meat quality of the carcasses.

According to the EU Council Directive of 1993 and the Regulation EU 1099/2009 on the protection of animals at the time of slaughter, animals brought into abattoirs for slaughter shall be a) moved and if necessary lairaged, b) restrained and c) stunned before slaughter. Animals must be restrained in an appropriate manner, so as to spare them any avoidable pain, suffering, agitation, injury or contusions. Animals must not be suspended before stunning or killing. However, poultry and rabbits may be suspended for slaughter provided that appropriate measures are taken to ensure that they are in a sufficiently relaxed state for stunning. Permitted methods for stunning are 1) captive bolt pistol, 2) concussion, 3) electro-narcosis and 4) exposure to special gas mixtures.

4.2 Eletrical stunning of poultry

Electrical stunning is based on the induction of a general epileptiform insult by the flow of an electrical current through the brain of animals, even if, unlike red meat species, poultry do not show grand mal epilepsy following this current flowing.

Two electrical stunning methods are generally used in poultry: *water bath stunning*, where the birds are immersed into an electrified water bath and *head-only stunning*, where the current is applied only to the head via a pair of electrodes. For both methods the depth and duration of unconsciousness depends upon the amount and frequency of currents applied

(EFSA, 2004). The frequencies used in modern electrical stunning systems of poultry ranges from 50 to 1500 or even 2000 Hertz. High frequency electrical stunning leads less pronounced contraction in the muscles and thus helps to prevent blood blemished meat that often lead to downgrading

Other possible carcass downgrading like broken bones and red wing tips are also reduced by using high frequencies. This is the reason for which higher stunning frequencies (> 300 Hz) have become more prevalent in slaughter plants (Bilgili, 1999).

The disadvantage of high frequency stunning is, that a shorter lasting stunning effect is produced, especially in the quickest recovering birds (Mouchoniere *et al*., 1999; Wilkins *et al*., 1998; Hillebrand *et al*., 1996) and extra care is needed in checking that birds remain insensible throughout the bleeding period (Gregory, 2007).

Regarding the waveforms of currents, the most frequently used under commercial conditions are direct currents (DC) and sine wave alternating currents (AC). By definition, in alternating currents the direction of the current is constantly changing, whereas in direct currents the direction of the current is constant. If the direction is constant but the magnitude is continuously changing it is commonly referred to as pulsed DC (pDC).

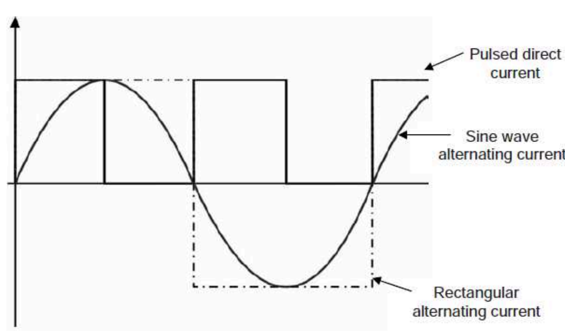


Figure 1. Illustration of different electrical waveforms

The form of an AC current can vary; some plants apply clipped or rectified waveforms, but a sinusoidal wave is the traditional form. More recently, modern commercial stunners are using a rectangular waveform.

The depth and duration of unconsciousness induced by an AC seems to be determined by the duration for which the current stays at maximum level within each cycle, otherwise known as the period (period =1/frequency). For example, electrical currents of 50, 400 and 1500 Hz sine-wave AC have periods of 20, 2.5 and 0.67 milliseconds, respectively. So, at a given current level the effectiveness of the electrical stunning depends upon the period of the current used and its decreases markedly when the period is below the threshold limit necessary to induce an epileptiform activity followed by a quiescent EEG. This effect could be due to the electrical-frequency dependent nature of the neurotransmitter release responses occurring in the brain. There are two pools of synaptic vesicles, a readily releasable pool at the plasma membrane and a reserve pool. The synapse can sustain high-frequency induced neurotransmitter release for many seconds without depleting vesicle pools. Moreover, high frequency stimulation also enhances significantly the rate of neurotransmitter replenishment.

When using a pulsed DC, the voltage employed to stun is expressed as the peak or average, since it flows from 0 to a peak voltage (unipolar). The amount of current delivered using a pulsed DC consists of *pulse width* of a DC, also known as duty cycle, and *space*. The pulse width : space ratio determines the relationship between the peak and average current of DC at any given frequency, according to the formula peak current =average current*period in milliseconds/pulse width in millisecond. Therefore, the peak current used to deliver an average current of 130mA of a 50 Hz pulsed DC, which is a period of 20 ms, will be 520 mA at 1:3 (130*20/5), 260 mA at 1:1 (130*20/10), and 173 mA at 3:1 (130*20/15) pulse width: space ratios. Based on the existing knowledge, it is suggested that, when using a pulsed DC, the pulse width : space ratio should be restricted o 1:1. In addition, it has been found that lowering the pulse width (e.g. by increasing the frequency) results in a less effective stun as in AC.

Sinusoidal AC is in general more effective at inducing stunning at lower current than pulsed DC. Raj, O'Callaghan, and Hughes (2006b and 2006c), in fact, have shown that a sine wave alternating current (AC) is more effective at producing EEG recordings indicative of unconsciousness and insensibility than the pulsed DC. The inefficiency of pulsed DC at inducing epileptiform activity is probably because the current flows in a positive direction only, whereas the AC flows both in positive and negative directions. In addition, compared with pulsed DC, sine wave AC has a relatively slower rate of voltage change and longer excursion distance (Raj, 2006). Direct brain cell stimulation studies have shown that AC current-induced electrical fields, affect the neuronal cell axis both parallel and perpendicular to the electrical field, whereas DC current induced electrical fields affect only the cell axis parallel to the electrical field. It is believed that electrical fields perpendicular to the cell axis are much more effective in affecting neuronal function than those parallel to the cell axes (Raj, 2006).

Despite their lower effectiveness, DC systems are used as they are perceived to cause less product degradation than AC systems. Several studies have been carried out to compare the effects of AC and DC stunning on carcass and meat quality of stunned broilers. Comparing AC currents of 200 Hz (100 mA) and 800 Hz (200 mA) with DC of 600 Hz (78 mA), alternating currents resulted in higher overall carcass downgrades than DC. For example, nearly 28% of minor fillets had significant haemorrhaging at AC 200 Hz compared with just over 7% at DC 600 Hz.

If a sufficient current amount, which is a function of the electrical impedance of the body of the bird, is administered a period of spike-and-wave epileptiform activity occurs. This epileptic process is characterised by rapid and extreme depolarisation of the membrane potential. This is followed by a period (of at least 30 seconds) of profoundly suppressed or quiescent EEG, indicative of spreading depression or neuronal fatigue in the brain (Schütt-Abraham *et al*., 1983a). In this phase, the release of monoamines and inhibitory amino acid neurotransmitters in the chicken brain seems to play a prominent role in the induction and maintenance of unconsciousness (Raj, 2003). The manifestation of a profoundly suppressed EEG, with the epileptiform activity reduced to less than 10% of the pre-stun level, and the abolition of Somatosensory Evoked Potentials (SEPs) following the stunning are suggested to be meaningful indicators of an effective electrical stun in chickens.

The duration of unconsciousness induced by a stunning method must also be longer than the sum of time that lapses between the end of stun and the time to onset of death. A minimum of 40s of unconsciousness would be ideal for all the stunning methods and species of animals. However, the onus of preventing resumption of consciousness thereafter relies on the efficiency of slaughter (bleeding) procedure; i.e. the prompt and accurate severance of blood vessels supplying oxygenated blood to the brain. In this sense, severing two common carotid arteries and two external jugular veins in the neck (ventral cut) has revealed more effective than unilateral cut (one common carotid an one external jugular vein severed).

Inducing cardiac arrest at the point of electrical stunning has welfare advantages (but is not a prerequisite) in chickens, because a delay between the end of stunning and neck cutting and the efficiency of neck cutting become less important. If cardiac arrest is induced at stunning, it results in cessation of the supply of oxygenated blood to the brain. This effect would certainly eliminate the potential problem of resumption of consciousness. When there is a cardiac arrest at stunning the birds quickly go limp after stunning, the wings and neck drop and the pupils dilate.

When a 50 Hz AC with sinusoidal wave form is used, the current necessary to induce cardiac arrest in 99% of chickens is 148 mA per bird (Gregory and Wotton, 1987).

In any case it has been demonstrated that the maximum percentage of chicken of cardiac arrests in chicken is achieved by using low frequencies values. As mentioned before, however, a commercial disadvantage of using conventional water bath stunners at low frequencies is that at currents greater than 105 mA per chicken (delivering for example 50 Hz AC) there is an increase in the incidence of haemorrhaging in the breast and leg muscles, broken bones in the carcass, and the appearance of carcass downgrading conditions (Gregory and Wilkins, 1989a, b).

4.2.1 Electrical water bath stunning

Electrical water bath stunning is the most common electrical stunning method used for poultry and requires upside down restraint in shackles. The water bath stunner is an open tank of water through which the birds are drawn as they are conveyed upside down by shackles along an overhead line. The water acts as the live electrode, and a metal bar which makes contact with the shackle usually acts as the earth electrode (see Figure 2). When the animals' heads touch the water the circuit is closed, causing the current to run through the head and body. As many as 20 birds may be making contact with the water electrode at any time, but, obviously, the exact number depends on the length of the water bath.

By means of an electronic display on the stunner, the stunning voltage can be regulated and adjusted to obtain the intended average current per bird at the water bath. This operation is aided by the presence of a digital ammeter.

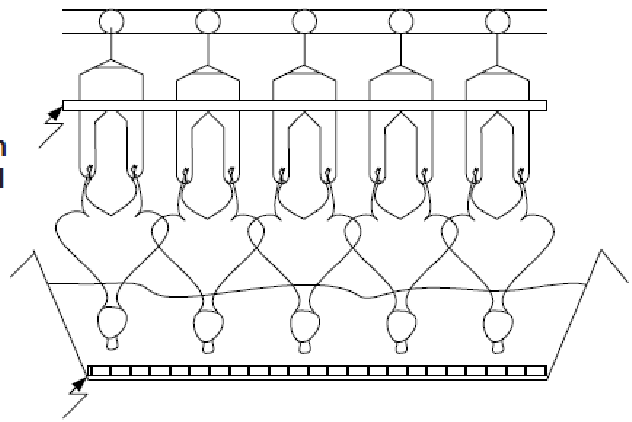


Figure 2. Schematic representation of electrical water bath stunning

In multiple-bird water bath stunning systems, the current flows through all birds at the same time and the electrical impedances of the birds form a form a constantly changing parallel circuit.

In an electric circuit, the relationship between current and resistance is described in ***Ohm's law***, usually presented as:

$$\boxed{\boldsymbol{V = I^*R}}$$

Where:

V = *voltage*, is expressed in *Volts*,

I = *current*, is expressed as *Amperes*,

R = *resistance* (or *impedance*) is expressed as *ohms* (Ω).

As for a conventional parallel circuit, the total current at the water bath is equal to the sum of the currents that flow through the individual broilers simultaneously immerged in the water bath. So, in the assumption that all the birds in the water offer the same resistance to the electrical current flowing, the current amount through each bird can be estimated dividing the total current (generally displayed on the ammeter of the stunner) by the number of birds simultaneously immerged in the tank.

According to calculations based on these principles, Sparrey *et al.* (1993) stated that broiler chickens have an effective impedance between 1,000 and 2,600 Ω, hens between 1,900 and 7,000 Ω, and ducks between 1,100 and 2400 Ω.

In a multiple bird water bath, when a constant voltage stunner is used, is possible that some birds receive more current than others, because the electrical impedance varies between birds (Raj and Tserveni-Gousi, 2000).

This is because, according to the law of electricity, birds showing high electrical resistance receive currents that are lower than necessary to render them unconscious, whereas birds having low electrical resistance receive more current than necessary to achieve effective stunning. Bird variation can be due to many factors, including body size, skull bone structure and thickness, body muscle and fat content, and plumage condition. It has moreover been suggested that the effective current per broiler varies between males and females due to the higher electrical resistance of female broilers (Rawles *et al*., 1995). Whether the feathers are wet, dry, or dirty, the depth of immersion and the tightness of shackles and their degree of fouling are also important factors (Bilgili, 1992; Boyd, 1994). Electrical variables also affect current flow. Mineral content, dirt, and brine concentration all affect the conductivity of the water bath (Bilgili, 1992; Boyd, 1994).

Stunning birds using a multiple-bird, electrified water-bath system is then a complex task, and it is extremely difficult, if not impossible, to adequately control the process (Raj, 2004).

To overcome the problem of variable electrical impedance in multiple-bird, water-bath stunners, constant-current stunners have been developed; however, these still have not been implemented in slaughter plants. These stunners control current flow through individual birds by electrically isolating each one to ensure that all birds in a multiple-bird, water-bath stunner receive the pre-set minimum current intended to achieve an adequate stun (Sparrey *et al*., 1993; Wilkins *et al*., 1999). However, because shackles are only 15 cm apart on the line and adjacent birds are in physical contact with each other, and because processing speed can be as high as 220 chickens per minute, there is considerable doubt that it is possible to electrically isolate each bird for long enough to deliver the pre-set current. As such, commercial application of these systems has been limited (Bilgili, 1999).

Based on the existing scientific knowledge (see for example Raj *et al*., 2006a), it can be suggested that the minimum current necessary to stun chickens would be 100, 150 and 200 mA per bird in a water bath supplied with up to 200, above 200 and up to 400, and above 400 and up to 1500 Hertz sinusoidal alternating current, respectively. For turkeys the minimum currents for the same frequency ranges were given as 250, 400 and 400 mA. These are also the minimum values indicated in the *Annex I* of the Regulation (EC) N. 1099/2009.

Other than the current amount, also the duration for which it is applied revealed a significant effect on the duration of unconsciousness. The application time under commercial conditions is usually around 10 seconds (Prinz, 2010b). A minimum application time of 4 seconds is indicated in the Regulation (EC) N. 1099/2009.

Following the technical requirements with electrical water bath is very important to achieve an effective stunning without unnecessary pain and suffering. To this aim, there must be secure and uninterrupted contact between the shackle and the earth (rubbing) bar. The height of the water bath must be adjusted according to the size of poultry. The electrodes in water bath stunners must extend to the full length of the water bath. There must be provisions such as electrically isolated entry ramps at the entrance to the water bath to prevent pre-stun electric shocks. For the same reasons the water must not overflow at the entrance of the bath. Birds' heads must be completely immersed in the water bath, preferably up to the base of their wings. Electrical devices must display visibly the total voltage and current delivered to the water bath and these should be appropriate to the waveform of the current used (EFSA, 2004; Schütt-Abraham, 1999). Voltage must be sufficient to ensure that every bird in the bath receives the recommended minimum current.

As mentioned before, electrical water bath stunning systems require the uncarting and shackling of live birds prior to stunning. It is likely that these handling procedures impose a considerable stress on the birds The negative impacts of shackling can be minimized as far as possible by limiting shackling time, using the appropriate size of shackle, preventing wing flapping (e.g. by using a breast comforter belt and using blue light).

Another important criticism of some water bath stunners is that they cause pre-stun shocks in the birds. There are four ways in which it occurs.

Firstly, in water baths that do not have an entry or exit ramp, water is continuously fed into the bath and it overflows from the entry or exit lip. There should in fact be no overflow at the entry lip, otherwise the birds will get an electric shock from this water (which will be electrically live).

Secondly, in water baths that are fitted with an entry ramp, the ramp becomes wet from water splashes out of the bath. If the ramp is not electrically isolated from the rest of the tank (e.g. by an air space), it will be live wherever there is a wet route to the water in the bath. Thus birds will get an electric shock when their heads make contact with the ramp and are drawn up it.

The third way is for the wing of the bird to dip into the water bath before the head. This is only a problem for slow line speeds, but it is a common feature in turkey plants because this species has a large wingspan and the wings hang below the head. It can be avoided by redesigning the entry ramp such that it holds the body of the bird back and allows the head, body and wings to be drawn together over the edge of the ramp into the water bath.

A fourth way in which broilers can get pre-stun shocks is when the rate at which their heads are immersed in the water is too slow and they recoil before being immersed. Another fault at some killing lines is that some birds miss the stunner altogether, either because they are runts and do not reach the water level, or because they are flapping their wings and raise their bodies as they pass over the bath. The problem of runts missing the water-bath can be avoided by not hanging them on the line with the other birds, but keeping them back and treating them separately.

4.2.2 Head only electrical stunning

In head-only electrical stunning the birds are restrained by hand, in a cone or shackles, between the legs or in a crush and the current is delivered by a pair of fixed electrodes, into which the head of the chicken is manually introduced (Wenzlawowicz *et al*., 2006).

This stunning method has not been implemented under commercial conditions where high throughput rates are required. The reasons for this are that: (1) the birds must be restrained to facilitate the correct placement of the stunning electrodes; (2) the electrodes must be kept clean and placed firmly on either side of the head so that they span the brain; and (3) neck cutting and severance of all the major blood vessels in the neck should be performed within 15 s of stunning to prevent the resumption of consciousness in the birds

Head-only electrical stunning induces flexion of legs followed by leg extension and wing flapping from the moment of current flows across the head. These are followed by tonic seizures as indicated by stiffening and arching of the neck, rigid extension of the legs, wings folded tightly around the breast and muscle tremor. During tonic seizure, eyes will be wide open (no blinking when touched) and rhythmic breathing will be absent. Return of eye reflexes and normal breathing precedes a return of consciousness (EFSA, 2004). One problem following head only stunning is the presence of severe wing flapping. This can represent an obstacle for prompt neck cutting. It can be addressed by either by prolonging the current flow through the brain or applying a so-called high frequency relaxation current through the spinal cord (Raj and Tserveni-Gousi, 2000; Hillebrand *et al*., 1996).

A minimum current of 240 mA for chickens and 400 mA for turkeys should be applied to the head for at least 7 seconds, when using a constant voltage stunner supplied with 50 Hertz alternating currents. Neck cutting must be performed within 15 seconds from the end of the stunning current (Gregory and Wotton, 1991; Gregory and Wotton, 1990a). With constant current stunners and low impedance electrodes, minimum currents increase with increasing frequency from 100 mA at 50 Hertz up to 200 mA at 1500 Hertz sinusoidal alternating currents. These currents have to be applied for at least 4 seconds (Raj and O' Callaghan, 2004).

4.2 Mechanical stunning

Poultry stunning mechanical devices have been developed specifically to kill, rather than stun birds (Hewitt, 2000). Because the skull bones are not ossified in poultry, both penetrating (percussive) and non-penetrative (concussive) devices induce severe structural damage to the brain and immediate death, rather than concussion of the brain (EFSA, 2004). Birds are restrained in cones, shackles, crushes or by hand and captive bolts must be fired perpendicular (at right angles) to the frontal bone (Raj and O'Callaghan, 2001). Bolt diameters between 5 and 6 millimetres and a length between 10 and 25 millimetres are reported to be effective in chicken. Following the stunning, visual evoked potentials (VEP) are immediately lost, but there can be severe wing flapping (Raj and Tserveni-Gousi, 2000; Hillebrand *et al*., 1996).

Non-penetrative captive bolt devices are used to stun and kill chicken and turkeys. They are fitted with a plastic or metal concussive head. The bolt head is fired with high velocity onto the head of the chicken or turkey and causes severe structural damage to the skull and brain.

The efficiency of this method depends largely on operators' skill and can hardly be standardized. So, it is mainly used in small scale slaughter plants, where prompt sticking is recommended and for casualty / emergency killing (HSA, 2005; HSA, 2004; Hewitt, 2000; Schütt-Abraham, 1995).

The development of captive bolt stunning has been negated in most species due to lack of means to prevent post-stun convulsions. Recently, a captive needle stunning method for broilers has been developed, in which air pressure is injected into the brains and partly directed to the spinal cord. The latter may prevent the convulsions. In broilers the air pressure stunning reduced post-stun convulsions to less than 13 % of the level of convulsions.

Figure 3. Mechanical stunning in turkey

The major welfare concerns related to mechanical stunning are the stress associated with live bird handling (restraint), the incidence of failure to shoot in the appropriate position, the prevalence of mis-stunning (number of birds requiring more than one shot) and delayed neck cutting and resumption of consciousness.

4.3 Gas stunning

One of the main welfare advantages of gas stunning is that the birds can be stunned in groups in their transport crates, so it avoids the pre-slaughter stress due to handle of removing them from the crates and hanging them on the shackles of the killing line when the birds are still conscious. Moreover, several studies have shown that electrical stunning may not cause an instantaneous stun in all broilers, even when applied in the prescribed manner, and so gas stunning would avoid the problem of some birds experiencing electric shocks before being stunned.

Poultry can be stunned with gas by exposing them to either *anoxic* (<2% oxygen by volume) *atmosphere* created with nitrogen, argon or other inert gases, or a *mixture of low concentration of* CO_2 (maximum 30% by volume) with nitrogen or argon leaving a maximum of 2% residual oxygen.

At present, the gas mixtures 90% Ar (argon) in air, 30% CO_2 (carbon dioxide) and 60% Ar in air are frequently used for poultry under commercial conditions.

The gases such as N_2 or Ar displaces O_2 from the air to be breathed, the gases such as CO_2 (in concentrations higher than 12%) affect directly the central nervous system.

Carbon dioxide has narcotic properties, it is a dense gas and so it is reasonably easy to contain. Moreover, it is a naturally occurring gas leaving no undesirable residues in the meat. However, CO_2 is aversive to poultry. It produces rapid unconsciousness when inhaled at high concentrations, but signs of asphyxia and behavioural excitation are observed due to occurrence of both hypercapnia and hypoxia. Moreover, it is an acidic gas and has been found to be painful and aversive in concentrations over 65%. Since the induction of unconsciousness with gas mixtures is a gradual process, the gas mixture should be non-aversive and the induction of unconsciousness should not be distressing to the birds (EFSA, 2004). For this reason CO_2 should not be used at high concentrations. Scientific evidence suggests that poultry seem to tolerate concentrations up to 30-40 percent carbon dioxide, whereas concentrations higher than 40 or 55 percent seem to cause pain or a high level of unpleasantness (EFSA, 2004).

Exposure of birds to 90% Ar in air or 60% Ar / 30% CO_2 in air results in an anoxic loss of consciousness.

The changes occurring in EEG and time to abolition of SEPs during the exposure to gas mixture have been used to determine the time to loss of consciousness (Wooley and Gentle, 1988; Raj *et al.*.1991, 1992, 1998). These seem to vary according to the oxygen and carbon dioxide levels in the mixture. Therefore, abolition of SEPs has been used as an unequivocal indicator of loss of consciousness, during exposure of chicken to various gas mixtures.

Exposure of chicken to anoxia created with 2% oxygen with argon results in loss of SEPs, on average, in 29 sec (Raj *et al.*, 1991). However, the duration of the unconsciousness provided by anoxia may not always be long enough to allow uncarting, shackling and bleeding of the birds.

Regarding CO_2 mixtures, it has been reported that exposure of chicken to 45% carbon dioxide in air results in loss of SEPs, on average, after 30 sec (Raj *et al*., 1990a). Increasing the concentration of CO_2 does not appear to reduce the time taken to lose consciousness.

Under anoxic conditions, depression of activity in the brain extends progressively from the telencephalon to the diencephalon and then to the mesencephalon. Anoxia results in suppression of the rostral reticula formation and therefore loss of consciousness and in suppression of caudal reticular formation and therefore onset of convulsions (with wing flapping)

Wing flapping is often observed in chickens before the onset of loss of posture when exposed to Ar or Ar / CO_2 gas mixtures. This pointed to the suggestion that wing flapping during immersion in the gas might be a response to an anoxic condition.

Wing flapping, however, can have a negative effect on carcass and meat quality, as it may result in broken bones and haemorrhaging.

Despite this problem, gas stunning is an effective method for stunning poultry, profiting especially from reduced handling and manipulation of live birds. Some concerns still apply such as unpleasant respiratory sensations which cannot be totally excluded, but the advantages of improved live bird handling more than counterbalance this risk.

Chapter 5
Assessment of unconsciousness

Recognition of the perception of pain by animals is a fundamental aspect of animal welfare and it is an important stage to assess the effectiveness of the stunning process.

Consciousness is defined in many different ways, but in general is associated with the awake state and the ability to perceive, interact and communicate with the environment and others (Zeman, 2001). The opposite state, that is, unconsciousness, is defined as: "a state of unawareness (loss of consciousness) in which there is temporary or permanent disruption to brain function. As a consequence of this disruption, the unconscious animal is unable to respond to normal stimuli, including pain" (EFSA, 2006).

Assessing unconsciousness is performed in a variety of ways, depending on species as well as the method of stunning.

The present chapter provides an overview of the measures that are usually used to assess unconsciousness in livestock with particular emphasis on those used for poultry.

5.1 Time to and duration of unconsciousness

Recommendations on the duration of stun-to-stick interval depend on different factors including the amount of current or concentration of gas used and the exposure time. When the stun is found not to be effective, the animal should be re-stunned as soon as possible. Animals that are conscious at time of the neck cut lose consciousness as a consequence of the severe decrease in cerebral blood flow leading to a rapid onset of disorganised brain function and thus unconsciousness (Mellor *et al*., 2009). Poultry, for example, lose spontaneous brain activity after on average 14 and 23 s when both carotid arteries are severed (Gregory and Wotton, 1984 and 1986). In cattle, however, consciousness after the neck cut is prolonged, as the vertebral arteries, which are not severed by the neck cut, supply blood to the circle of Willis and play a direct role in the blood supply to the brain (Baldwin and Bell, 1963). Cattle lose spontaneous brain activity 75 ± 48 s post neck cut (range 19 to 113 s), but Newhook and Blackmore (1982) suggested possible intermittent sensibility for up to 123 to 323 s after slaughter in cattle (Daly *et al*., 1988). The time to loss of consciousness in non-stunned animals, emphasises the need to verify unconsciousness after stunning.

5.2 Assessing unconsciousness in livestock

Unconsciousness, caused by temporary or permanent disruption to the brain, is generally assessed by the observation of behavioural indicators, which are internally coordinated responses to internal or external stimuli (Levitis *et al*., 2009). They include reflexes originating from the brain stem (e.g. eye reflexes) or spinal cord (e.g. pedal reflex) and behavioural indicators such as loss of posture, vocalisation and rhythmic breathing. In an experimental set-up, the assessment of brain activity as presented in an EEG, derivatives of the EEG, and evoked potentials can be used to assess unconsciousness.

5.2.1 Reflexes

Reflexes are automatic, stereotyped movements that are produced as the direct result of a stimulus and are mediated by the central nervous system (Carlson, 2007). Central reflexes are indicators of consciousness that are linked to functioning of the brain stem or spinal cord. Brain stem reflexes are regulated by 12 pairs of cranial nerves that enter and exit the brain and are not under cortical control. Two cranial nerves (I and II) enter from the forebrain and the other nerves (III to XII) enter and exit from the brain stem (Carlson, 2007; Rubin and Safdieh, 2007). Brain stem reflexes that are used to assess unconsciousness after stunning in livestock are:

- *cornea or blinking reflex*,
- *palpebral reflex,*
- *pupillary light reflex* and
- *threat reflex*.

The *corneal reflex* causes involuntary blinking of the eyelids in response to stimulation of the corneal and is in general the last reflex to be lost in anaesthetized animals (Dugdale, 2010).

Figure 4. Assessment of corneal reflex in a broiler chicken

The *palpebral reflex* also results in blinking as a response to touching the medial canthus of the eye and disappears earlier than the corneal reflex in anaesthetised animals. Both the corneal and palpebral reflex require a functional afferent cranial nerve V (trigeminal) and efferent cranial nerve VII (facial) and the relevant eye muscles to function adequately (Adams and Sheridan, 2008). The corneal (blinking) reflex may be difficult to test when the eyelids are shut, whereas the nictitating membrane reflex can be observed in closed eyes when the eyelids are gently opened (Erasmus *et al.*, 2010).

The *pupillary light reflex* is tested by letting light fall on the eye and observing whether the pupil adapts to it. The reflex is controlled by cranial nerves II (optic) and III (oculomotor) and is not considered a reliable reflex during exsanguination, as exsanguination interferes with the blood supply to the retina (Blackman *et al*., 1986). When testing the threat reflex, an object (finger or pencil) suddenly approaches the eye and a conscious animal will close its eye or withdraw the head. This reflex requires a functional efferent cranial nerve VII (facial) and integration of the motor cortex, but is not often applied, as it requires the eye to be open. *Focused eye movement*, which is not a reflex, is considered a definite sign of consciousness, as it needs cortical activity for perception and controlled motor activity from the eyeball muscles (Grillner *et al*., 2008; Vogel *et al*., 2011). It has to be pointed out that positive eye reflexes alone do not necessarily indicate consciousness, as positive brain stem reflexes might occur on the basis of residual brain stem activity and do not distinguish clearly between consciousness and unconsciousness (Anil, 1991). This especially holds true for animals that are electrically stunned, which was documented as early as 80 years ago (Roos and Koopmans, 1936; Blackmore and Delany, 1988; von Holleben *et al.*, 2010). In both sheep and calves, brain stem reflexes were present long after electrical stunning, even though the EEG was suppressed or iso-electric (Anil, 1991; Anil and McKinstry, 1991). So, cranial nerve reflexes can be good indicators for impaired midbrain or brain stem activity, but only work reliably in one way: when absent, it is very likely that the animal is unconscious, but when they are present, the animal is not necessarily conscious.

Spinal reflexes include *stretch* and *flexor reflexes*. The stretch reflex, a monosynaptic reflex, is the most basic reflex and is elicited by activation of muscle spindles (receptors within muscles. It is involved in maintaining muscle tone and plays an important role in control of posture, but it is not used to evaluate the state of insensibility as it does not involve the brain (Carlson, 2007). Conversely, the flexor reflex (pedal or withdrawal), a polysynaptic reflex, involves activation of nociceptors and is used to assess unconsciousness (Anil, 1991; Erasmus *et al*., 2010). An example of a flexor reflex is the pain withdrawal reflex, which is elicited by applying a painful stimulus to the animal, such as a nose or ear prick. In a survey on expert opinion, the pain withdrawal reflex was ranked high, and thus valued highly, as an indicator to assess unconsciousness after all types of stunning (Gerritzen and Hindle, 2009).

The *pedal reflex* is indicated by withdrawal of the foot in response to pressure applied to the toes. Absence of the pedal reflex may not be indicative of insensibility under all circumstances; animals that are paralyzed may not exhibit pedal reflexes but may be fully sensible. This reflex is often used for assessment of depth of anaesthesia, although it is not consistent in all species.(Erasmus *et al.,* 2010), but is only occasionally applied in livestock after stunning, as all spinal reflexes are difficult to assess when animals exhibit convulsions or body movements (Tidswell *et al*., 1987). This especially holds true for animals that are physically stunned, for example with captive bolt stunning, when there is lack of inhibition from the brain and spinal reflexes may occur more vigorously (Blackmore and Delany, 1988). Again, electrically stunned animals may exhibit this reflex long after losing consciousness and the reflex may occur more vigorously when the animal is handled (Blackmore and Newhook, 1982).

The *righting reflex* refers to any reflex that tends to bring the body into its normal upright position. It is often assessed when animals are removed from the stunning box or are hung to the bleeding rail and is also referred to the *head righting reflex*. This reflex is also difficult to assess when animals exhibit convulsions or involuntary body movements (Blackmore and Newhook, 1982; Anil, 1991).

Table 2, shows an overview of the different brain stem and spinal reflexes used to assess unconsciousness after stunning.

5.2.2 Behavioural indicators

Loss of posture, i.e. the inability of the animal to remain in an initial standing or sitting position, is considered a valuable indicator as it is often the first sign to be lost after successful stunning and indicates that the cerebral cortex is no longer able to control posture (Raj et al., 1992; Raj and Gregory, 1996; Llonch *et al*., 2013). Both mechanical and electrical stunning should lead to immediate collapse (AVMA, 2013).

Nystagmus, involuntary rapid horizontal eye flickering, is caused by damage to the vestibular, labyrinthine or central nervous system and is more present in cattle that have a shallow depth of concussion following captive bolt. Nystagmus may also occur as a result of electrical stunning (Grandin, 2002), but in CO_2-stunned pigs, nystagmus was not observed once (Atkinson *et al*., 2012).

Table 2. Reflexes used to assess unconsciousness after stunning

Reflex	Definition	Presence in animals that are[1]		Based on	Remarks
		conscious	unconscious		
Brain stem reflex	originate from brain stem			Functional cranial nerves originating from the brain stem	Reflexes may be present in animals that are unconscious, depending on the method of stunning (Gerritzen and Hindle, 2009).Absence of these reflexes though are considered valuable indicators for assessing unconsciousness (von Holleben et al., 2010) Cannot be tested when seizures occur (Blackmore and Delany, 1988).
Corneal reflex	Involuntary blinking in response to stimulation of cornea	+ (−)	− (+)	Functional cranial nerves V and VII and eye muscles	One of the most commonly used reflexes after stunning. In general the last reflex to be lost in anaesthetised animals (Dugdale, 2010). May be present after electrical stunning, but never after effective captive bolt stunning (Roos and Koopmans, 1936; Gregory and Shaw, 2000)
Palpebral reflex	Involuntary blinking in response to touching the medial canthus of the eye	+ (−)	− (+)	Functional cranial nerves II and III and eye muscles	Disappears earlier than the cornea reflex in anaesthetised animals (Dugdale, 2010).
Pupillary light reflex	Narrowing of the pupil in response to light that falls on the retina	+	−	Disappears earlier than the cornea reflex in anaesthetised animals (Dugdale, 2010)	Considered of little value during exsanguination, as the blood supply to the retina is restricted during this period (Blackman et al., 1986) Pupillary dilatation is considered a sign of total brain dysfunction (Blackman et al., 1986) May be absent in paralysed, though conscious animals (Blackmore and Delany, 1988)
Threat reflex	Involuntary blinking or withdrawal of the head in response to bringing a finger or hand with speed towards the eye of an animal	+	−	Functional cranial nerve VII, eye muscles and integration with motor cortex	Cannot be tested when the eyes are closed
Spinal reflex	Reflexes that originate from the spinal cord			Require a functional spinal cord, but do not necessarily require cerebral coordination.	May occur more vigorously when there is lack of inhibition from the brain (e.g. captive bolt stunning; Blackmore and Delany, 1988).

Reflex	Definition	Presence in animals that are[1]		Based on	Remarks
		conscious	unconscious		
Pain withdrawal reflex	Withdrawal of the body part that has had a painful stimulus applied to	+ (–)	– (+)		In a survey on expert opinion, the pain withdrawal reflex was ranked high, and thus valued highly, as an indicator to assess unconsciousness after all types of stunning (Gerritzen and Hindle, 2009)
Pedal reflex	Withdrawal of the foot in response to pinching (the skin between) the toes of an animal	+ (–)	– (+)		Difficult to assess when convulsions occur. Not easy to perform in all species. Mainly used in poultry
Righting reflex	Bringing the body into its normal position when taken out of its normal upright position	+ (–)	– (+)		Difficult to assess when convulsions occur (Blackmore and Newhook, 1982; Anil, 1991)

[1]*Presence and absence of reflexes are presented as follows: + = present, – = absent, (+) = may be present, (–) = may be absent.*

It is stated that under no circumstances should a stunned animal vocalise, as *vocalisation* after stunning indicates consciousness and probably distress and pain (Grandin and Smith, 2004; Gouveia *et al*., 2009). A large network of brain regions is involved in the production of vocalisations, including the frontal lobe and primary motor cortex and vocalisations are considered a conscious response (Carlson, 2007). The involuntary passage of air along the vocal cords, however, may cause sounds that can be mistaken for vocalisations. Absence of vocalisations on the other hand, is certainly no guarantee for absence of pain or distress, as the occurrence of vocalisations also depends on the species. A sheep often does not vocalise when injured, where a pig will scream loudly (Broom, 2001; EFSA, 2004).

Grandin, (2002), believes an animal to be unconscious when it shows a *limp head* and *protruding tongue*. The tongue is controlled by nerve XII (hypoglossal) and when relaxed this may indicate loss of cranial nerve function. A study by Gregory *et al*., (2007) showed that a protruding tongue was not associated with depth of concussion after captive bolt stunning, but was proposed as indicator following exsanguination, when 40% of the cattle had a protruding tongue while hanging on the bleeding rail. Similarly, *relaxation of the jaw* may be taken into account, but can be observed in conscious animals (Gregory *et al*., 2009). Both jaw relaxation and tongue protruding are not used as single indicators to assess unconsciousness, but can support other indicators of unconsciousness (Grandin, 2002; von Holleben *et al.,* 2010). Beside the important role regarding consciousness, the brain stem also houses the regulatory centres for respiratory and circulatory systems. *Rhythmic breathing* movements after stunning indicate that the corticospinal, ventral and lateral columns of the spinal cord are still intact and may thus indicate consciousness (Mitchell and Berger, 1975). The presence of rhythmic breathing after stunning is generally accepted to indicate that an animal may not be fully unconscious and is thought to be one of the first signs of recovery after CO_2 and electrical stunning (Gerritzen and Hindle, 2009; Anastasov and Wotton, 2012). In captive bolt stunned cattle, rhythmic breathing immediately disappears after an effective shot because of axonal injuries to the brainstem (Finnie *et al*., 2000). The *occurrence of convulsions*, observed as uncontrolled movements of the body, indicates effective stunning in electrical or mechanical stunned animals, but also occur in unconscious animals that are gas stunned (Adams and Sheridan, 2008; Marzin *et al*., 2008; von Holleben *et al*., 2010). These convulsions are thought to be incompatible with consciousness due to the absence of higher motor control (Lambooij, 2004). They can, however, sometimes be mistaken for rhythmic breathing, as they can occur as almost rhythmic body movements (Wotton and Sparrey, 2002). *Gagging* refers to low-frequency inhalations with the neck positioned towards the front legs and occasional emission of sounds similar to snoring and is considered an indicator of deep unconsciousness (Rodríguez *et al*., 2008). *Gasping* is seen when an animal takes deep breaths through an open mouth and is considered an indicator of onset of breathlessness during CO_2 stunning, which continues long after loss of consciousness even when brain activity is no longer recorded, but may also occur after electrical stunning (Blackmore and Petersen, 1981; Newhook and Blackmore, 1982; Grandin, 2013). Interpretation of all individual indicators mentioned above can be doubtful unless supported by other information (Blackmore, 1984; Gerritzen and Hindle, 2009; Anastasov and Wotton, 2012).

When assessing unconsciousness, pathophysiology of the consequences of the stunning method should be taken into account, as applicability and reliability of the different indicators vary per stunning method. When using physically stunning, for example captive bolt, the most important indicators are posture, righting reflex, rhythmic breathing and the corneal or palpebral reflex that should all be absent when the animal is unconscious. Spinal reflexes are difficult as a measure of unconsciousness with this type of stunning, as they may occur more vigorous.

For stunning methods that do not physically destroy the brain, for example, electrical and gas stunning, most important indicators are: brain stem reflexes, posture, righting reflex, natural blinking response, rhythmic breathing, vocalisations and focused eye movement.

5.2.3 Brain activity (EEG)

When monitoring brain functioning, activity can be presented in an electroencephalogram (EEG), which displays electrical activity derived from electrodes attached to various locations on the surface of the head. The EEG is considered the most objective method for assessing unconsciousness and reflects the sum of underlying electrical activity of populations of neurons supported by glia cells (Murrell and Johnson, 2006). There are four different types of wave patterns in the EEG that can be distinguished based on their respective frequencies and that are related to the state of consciousness:

- ✓ δ (0 to 4 Hz) waves,
- ✓ θ (4 to 8 Hz) waves,

- ✓ α (8 to 12 Hz) waves and
- ✓ β (>12 Hz) waves.

Both delta and theta (slow wave) activity is related to sleep or reduced consciousness. Delta waves together with theta waves, for example, predominate in human infants during the awake state. Alpha activity is prominent in subjects that are conscious, but mentally inactive (closing eyes and relaxation) and beta waves are associated with active movements and increased alertness (Kooi *et al*., 1978; Niedermeyer *et al*., 2011).

Depending on stunning method, the EEG shows a characteristic pattern of change when animals lose consciousness.

Four stages of EEG can be distinguished during the process of stunning and slaughter and are related to the level of consciousness, namely: *active, transitional, unconscious and iso-electric (flat) EEG*. In the first (active) stage, normal awake activity is recorded with high frequency, low amplitude waves, indicating the animal is conscious. In the second (transitional) stage, the amplitude of the EEG increases together with a decrease in frequency. When these changes become more profound, the animal is considered unconscious. When loss of consciousness progresses, the EEG turns iso-electric and brain activity is no longer recorded (Gibson et al., 2007; McKeegan et al., 2007). The exact moment when unconsciousness sets in, based on the EEG, is difficult to determine as changes are often gradual. It is generally accepted, however, that establishing an iso-electric EEG pattern after stunning is characteristic for unconsciousness. SchüttAbraham et al. (1983) recommends a minimum of 30 seconds iso-electricity as a parameter for adequate stunning.

5.2.4 Evoked responses

The EEG recording is also used to assess unconsciousness by way of generating evoked responses. Evoked responses arise as a result of electrical activity generated in specific areas of the cerebral cortex, mid brain and brainstem (Schneider and Sebel, 1997; Grover and Bharti, 2008) in response to a particular stimulus as somatosensory, auditory and visual evoked responses. These responses are elicited by exposing the animal to a stimulus (visual, somatosensory or auditory) and then recording the resulting changes in brain activity using EEG telemetry. The absence of brain activity in response to the stimulus indicates that the animal is insensible.

Evoked responses are frequently used as additional indicators to assess unconsciousness next to behavioural indicators, and have been applied in sheep, cattle, poultry and pigs. No correlations, however, have been calculated for the presence or absence of evoked potentials and presence or absence of behavioural indicators. Evoked potentials might in this way provide additional support for the use of certain behavioural indicators. As for now, evoked responses are only used in experimental set-ups. Rapid changes in consciousness are difficult to observe with evoked potentials, as repeated stimulation and averaging of data (EEG) is needed to see these changes (Beyssen et al., 2004).

Differences in time to loss of consciousness based on the loss of spontaneous EEG or evoked responses have been observed in multiple studies. In hens stunned with different gas mixtures, evoked responses were observed to disappear ~15 s after the EEG became suppressed, but almost 30 s before the occurrence of an iso-electric EEG (Raj et al., 1991 and 1992). In poultry slaughtered by nine different methods, all without prior stunning, spontaneous brain activity was lost after 23 to 233 s, where visual evoked potentials were lost after 90 to 349 s (Gregory and Wotton, 1986). The loss of somatosensory evoked potentials was also recorded before an iso-electric EEG, but after a suppressed EEG in gas stunned turkeys (Raj and Gregory, 1993). The presence of an evoked response implies that the afferent pathways to the higher brain centres are intact, but not necessarily that the animal is aware of the stimulus (Raj et al., 1991). Visual evoked potentials have been observed in, for instance, anaesthetised animals (Gregory and Wotton, 1986; Gregory, and Wotton, 1989). Conversely, the absence of evoked potentials may not always guarantee unconsciousness (Anil et al., 2000). Gregory and Wotton (1990) looked at the effects of multiple electrical stunning currents on spontaneous physical activity and evoked responses and found that the loss of somatosensory evoked potentials indicated a deeper level of unconsciousness than absence of neck tension. All these studies show that the use of different methods to assess unconsciousness may lead to different findings regarding the time to loss of consciousness. The use of absence of evoked responses or iso-electric EEG, may provide more conservative times to loss of consciousness compared with loss of spontaneous EEG. Furthermore, the EEG and evoked responses measure cortical activity and do not provide direct measures of brain stem function, which includes control of respiration and heart rate. More commonly, the absence of brain stem and spinal reflexes is used as an indicator of insensibility (Erasmus *et al*., 2010).

5.2.5 Difficulties in the use of EEG

Though the EEG may be considered most objective when assessing unconsciousness, there are some disadvantages to its use. First, there is no golden standard for the way in which the division of stages of consciousness is described and this also limits the use of brain function monitors in differentiating between consciousness and unconsciousness, especially during transitional stages (Alkire *et al*., 2008).

Second, it is difficult to compare EEG values between species and individuals, because of animal variation caused by electrode placement, skull thickness and differences between equipment. Third, the EEG can be influenced by artefacts that are animal related (eye or muscle movements) or technical related (cable movements, impedance fluctuation or 50/60 Hz interference) (Teplan, 2002). Experimental controlled situations provide a significantly better environment to limit these artefact sources than slaughter plants. These artefacts, however, limit possibilities for EEG application as an evaluation method in slaughter plants at this stage.

5.3 Assessment of insensibility in poultry

There is no “gold standard” for assessing insensibility in poultry, and different researchers use different methods.

Avian brains possess structures analogous to those necessary for consciousness in mammals. The particular areas of the brain involved in consciousness and arousal include the brain stem, thalamus, and cerebral cortex. Within the central core of the brain stem, a collection of neurons known as the reticular formation forms ascending and descending neural networks. The ascending networks form the ascending reticular activating system, which projects through the thalamus and hypothalamus and into the basal forebrain and is responsible for consciousness. Indeed, electrical activation of the reticular activating system results in consciousness and arousal in mammals and birds. The descending neural networks, on the other hand, project to the spinal cord, where they facilitate or inhibit sensory and motor activity. The reticular formation not only is responsible for consciousness, but also acts on other parts of the central nervous system, such as cranial nerves in the brain stem and nerves in the spinal cord, and influences movement and autonomic functions.

The insensibility results from lesions to the reticular formation, lesions to pathways that connect the reticular formation to the cerebral cortex, and direct injury to the cerebral cortex or brain stem. The state of unconsciousness is also produced when there is physical disorder of brain activity through depolarization of nerve cells, which is caused by electrical stunning, hyperpolarization of specific neurons in the thalamus by general anaesthetics, and concussion, which is caused by rapid acceleration of the head.

As for other animals, the measures that are used to assess the state of insensibility after stunning in poultry include the EEG, evoked responses, brain stem and spinal reflexes and other behavioural measures, such as jaw and muscle tone.

5.3.1 Use of EEG in poultry

The EEG recordings of chickens following electrical stunning often differ from those of mammals in that the epileptic activity more closely resembles a petit mal seizure (Gregory & Wotton, 1987), a milder form of epileptic attack in humans (Gregory, 1986). These kinds of seizures are not associated with immediate unconsciousness in humans (Goldie & Green, 1961; Porter & Penry, 1973), and this is one of many lines of evidence to suggest that electrical stunning does not produce immediate unconsciousness in all birds (Gregory, 1986; Gregory & Wotton, 1987; Boyd, 1994; Raj, 2003). However, because the brain of a chicken responds to electrical stunning differently from the brain of a mammal (Raj, 2003), the subjective experiences of a bird and a mammal may also differ during a petit mal seizure.

For both mammals and birds, the occurrence of an *epileptiform EEG* recording, followed by a *quiescent phase*, is thought to be the best available evidence of unconsciousness and insensibility and is used in studies of electrical stunning as a measure of the effectiveness of the stun (Raj, 2003; Raj, O'Callaghan, & Knowles, 2006a). Somatosensory evoked potentials (SEPs) are abolished during the manifestation of these two EEG patterns and are a complementary measure of brain function. When passed through an electrical water-bath stunner, birds may show seizures, visually indistinguishable from effectively stunned birds, without the manifestation of epileptiform activity in the EEGs. The amount of current necessary to induce an epileptiform EEG, indeed, is more than the amount necessary to induce seizures (Schütt-Abraham *et al*., 1983, Raj *et al*., 2006a).

In view of the fact that both effectively stunned birds and ineffectively stunned birds exhibit tonic seizures, it is more than likely that muscular paralysis, rather than unconsciousness and insensibility, is induced by prolonged application of inadequate current parameters. This induction of seizures in conscious birds would obviously cause pain and suffering.

Raj and O'Callaghan (2004a and 2004b) calculated the total power content of EEG patterns to establish the state of vigilance and consciousness in broiler chickens. According to their studies, a total power content of less than 10% of the pre-stun level in the frequency band 2-30 Hz is considered characteristic of iso-electricity and *deep unconsciousness*. On the other hand, the lack of activity in a smaller band (from 13 to 30 Hz) in stunned chickens has been interpreted as an "unequivocal *loss of sensibility*" (Raj and O'Callaghan, 2004a and 2004b).

5.3.2 Indicators of unconsciousness and death in poultry

Several methods have been used in assessments of insensibility after stunning or killing in poultry.

The absence of neck tension has been used as an indicator of insensibility with gas killing methods, captive bolt shooting, and electrical stunning. Absence of neck tension has been estimated to occur at the same time as suppression of EEG activity. The onset of sustained eye closure has been shown to occur at the same time or shortly after loss of somatosensory evoked responses and has been used as an indicator of insensibility when gas killing methods were used.

Effective stunning leading to death should result in loss of reflexes and also complete cessation of breathing. The resumption of rhythmic breathing is in fact one of the first indications that sensibility is returning. However, the simultaneous occurrence of neuromuscular spasms may make difficult to examine breathing when physical euthanasia methods are used.

Another sign to determine the time of death in poultry after killing also include the occurrence of feather erection, together with tonic muscle spasms that are followed by complete muscle relaxation. In some studies, in fact, the time at which the neuromuscular spasms cease has been used as an indicator of when irreversible brain failure occurs. These neuromuscular spasms are typical reflex reactions in animals that are stunned with physical methods, and they also occur after decapitation when not preceded by stunning and during rapid induction of anoxia. In birds, neuromuscular spasms consist of a clonic phase, characterized by vigorous wing-flapping, and a tonic phase, characterized by stillness, with legs and wings outstretched, which is followed by final paddling motions leading to relaxation and death.

Neuromuscular spasms resulting from electrical stunning are generally considered to be incompatible with sensibility. However, as mentioned in the previous paragraph, also ineffectively stunned birds may exhibit convulsions that may occur before loss of posture and insensibility. Moreover, it is not always possible to distinguish between neuromuscular spasms that are incompatible with sensibility and other involuntary muscle contractions (myoclonic jerks). Therefore, the onset and presence of convulsions do not indicate insensibility, but the sustained and complete absence of convulsions when physical stunning and killing methods are used may be indicative of ineffective stunning and killing.

Insensibility and effective stunning can generally be recognized by the absence of eye reflexes, such as the nictitating membrane or corneal reflex, and the absence of breathing. In addition, in an effective stunning there should be no response to painful stimuli (such as comb or toe pinching), there should be no neck muscle tension and vocalization should be absent because it may be indicative of pain or distress. It has to be pointed out, however, that the comb pinch response may be absent also in inadequately stunned birds, when an electrical water bath stunning is used (Schütt-Abraham *et al*., 1983).

The corneal reflex is the last to disappear and is indicative of complete insensibility. As other brain stem reflexes, however, positive corneal reflex might occur on the basis of residual brain stem activity and does not distinguish clearly between consciousness and unconsciousness. So, when absent, it is very likely that the animal is unconscious, but when it is present, the animal is not necessarily conscious. Corneal reflex, for example, can also be present during cardiac arrest.

Chapter 6
Animal welfare and meat quality in poultry

The present chapter gives an overview of the main welfare problems that affect poultry industry (including those at the abattoir) and their effect on both carcass and meat quality.

6.1 Welfare concerns in poultry and effect on carcasses and meat

6.1.1 Problems at livestock and prior to the abattoir

Leg disorders are one of the most common welfare problems in poultry (Kestin *et al*., 1992). In varying degrees they cause pain for the birds, and in some cases they cause wasting, dehydration and death. In the worst cases of lameness the birds have a *Staphylococcus aureus* infection, which they probably acquire through the navel when they are chicks. This results in structural changes in the femur, which becomes weakened, and often the bird is debilitated and growth is reduced. This condition is known as osteomyelitis. In severe forms the bird cannot walk or it can only walk to the feeders and drinkers by taking a few paces at a time. Overall, between 1 and 5% of a flock may be affected when the birds reach slaughter age. At the slaughter, the weakened femur can affect the quality of the thigh portions that are produced in the portioning line. If the femur breaks during automatic thigh bone removal in the portioning line, part of the femur is left in the meat as a contaminant.

The birds affected by leg disorders are often reluctant to walk for long and they have an unsteady, strutting gait. In a proportion of these birds walking is a painful experience, but their growth is not necessarily affected. The angulation of the leg creates problems at the processing plant. It makes it difficult, for example, to insert the bird properly into the shackle without causing pain.

The development of non-infectious leg disorders can be slow down by giving the birds less feed or by reducing the nutrient density of the feed, in fact this helps to limit weight-bearing during a critical period in their leg growth. For this purpose, it is important to limit the feed intake during this early period is to control the lighting pattern because when the lighting is switched on, birds usually stand up and have a feed. By controlling the frequency with which the lighting comes on and off, feed intake and leg disorders can be reduced.

It is also important to avoid *overstocking* problem in the farm because at high densities the birds are less active and they are more prone to skin as well as leg disorders, they walk less but there is more interference between birds. When a bird walks to a feeder or drinker in an overcrowded shed, it is likely to bump into other birds that are squatting on the floor. So in this case, the birds that are moving may scratch the squatting birds with their claws. This results in scabby hips which form unsightly blemishes in the carcasses and lead to carcass downgrading. As stocking density rises and the birds compete more, feed intake per bird starts to decline (Bolton *et al*., 1972; Shanawany, 1988). This in turn leads to reduced growth rate per bird.

Keeping broilers in *dim lighting* reduces their activity, it does not necessarily compromise their walking ability and it is often used as a way of limiting the amount of energy they use in taking exercise. Dim lighting is apt to make the birds more flighty when they are taken out of the sheds and experience bright daylight for the first time. Flightiness causes red wingtips and bruising through wing flapping and this leads to downgrading of the carcasses.

As the birds grow, they fill the available space in the shed. As they get taller, they knock against the drinkers which are suspended from the roof. This causes spillage of water on to the litter, making it sticky and the birds dirty, and the moisture encourages bacteria in the litter to produce ammonia, which fouls the atmosphere in the shed. These problems are managed by raising the drinkers as the birds grow, increasing the clearance above the floor. The litter in the rearing shed has to be managed as it has some important effects on bird quality. Litter moisture content is mainly controlled by regulating the rate of air flow through the shed. This becomes more difficult at high stocking densities, as the birds form a windproof layer over the litter, which fails to dry. If the birds sit on wet litter they are prone to developing hock burn (scab on the hock).

Lame birds are more likely to develop hock burn because they spend more time squatting on the litter. Similarly, large lame birds are more likely to develop breast blisters, especially on hard floor surfaces. When a bird squats on the floor, 59% of its weight is borne by its keel; if the surface is hard, watery cysts can develop where the keel presses against the floor. They are a cause of carcass downgrading, because they are unsightly. Breast blisters occur below the skin, whereas breast burns occur in the skin. These are patches of dead skin tissue over the keel where it has been in contact with wet or badly compacted litter. The corrosive effect of the faeces and urine causes localized burning of the skin, which may become infected through prolonged exposure to wetness. It is more common in males than females and in birds kept at high stocking densities. Less commonly, the follicles in the breast feather tracts become infected, resulting in an unsightly folliculitis, which is also a cause of carcass downgrading. All these carcass quality problems are avoided by not overstocking, by managing the broiler shed litter correctly and by avoiding situations that lead to diarrhoea in the birds.

Modern broiler strains have been bred for growth rate and breast conformation. Compared with broilers of the 1950s, they are faster growing and they convert feed into live-weight more efficiently, but they are more prone to dying early in life as well as developing leg disorders or stress-induced myopathies (Havenstein *et al*., 1994).

Feed should be stopped before the birds are slaughtered, to allow the gut to empty. This should help to reduce the likelihood of gut contents leaking or spilling on to the carcass, but on the down-side it will assist the establishment of Salmonella within the caeca (Moran and Bilgili, 1990).

The optimum fasting period that allows adequate gut emptying without excessive loss of carcass weight is 10 hours.

The transport has also an important effect on welfare and carcass quality. When birds are transported for 6 hours or longer, the ultimate pH of the thigh meat can be raised (Warriss *et al.,* 1993). If the $pH_{15\ min}$ of breast meat is 6.5 or higher, the meat is more prone to bacterial growth (Stawicki *et al*., 1976). There is little doubt that birds are stressed when they are caught and loaded into transport crates before slaughter. This has been shown by measuring the plasma corticosterone concentrations in plasma (Figure 5) (Knowles and Broom, 1990). When birds were caught and carried in the normal manner whilst held upside down by the legs, the stress response was greater than that in birds caught and carried gently in an upright position. Catching teams are expected to load birds at a rate of 1000 to 1500 birds per person per hour, and it is inevitable that at these rates there will be some emotional stress in the birds.

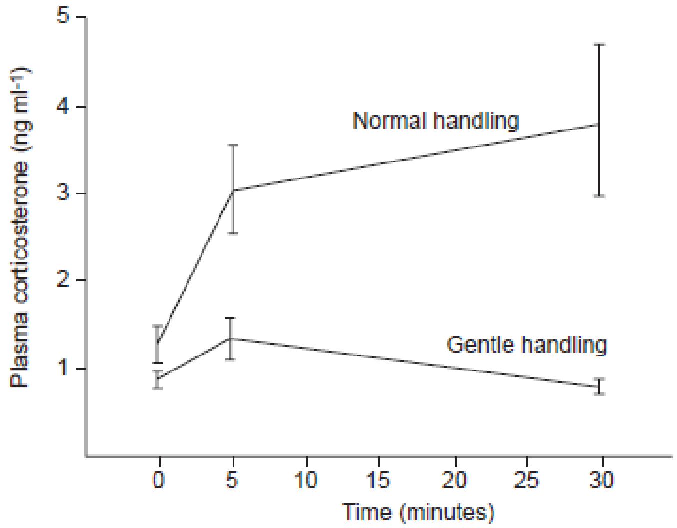


Figure 5. Plasma corticosterone response in chickens handled normally or gently

During the catching procedure there is also a risk that the birds will damage each other. If the flock gets alarmed the birds migrate towards one end of the shed, where they may pile up on each other and cause backscratching and death from smothering. It is important not alarming the birds so the lighting is turned off or kept to a low level and noise is controlled.

Normally the birds are held by one leg as a bunch of birds in each hand. If one or more birds start flapping they twist at the hip, the femur detaches, and a subcutaneous haemorrhage is produced which kills the bird. This is more common in larger birds and quite often the detached femur is forced into the abdominal cavity, where it ruptures an air sac, allowing blood to enter the lungs. Dead birds that have a dislocated hip often have blood in the mouth, which has been coughed up from the respiratory tract.

Sometimes this damage is caused by too much haste on the part of the catchers.

There are two important forms of *ante-mortem bruising* in poultry*: breast bruising* and *red wingtips.*

Breast bruising can occur during transport on rough roads and in trucks with inadequate suspension, but it can also occur when heavy birds, especially turkeys, are loaded into the transport crates. If they are thrown in and the breast hits the edge of the transport crate, a breast bruise can develop.

Red wingtips can be due to violent flapping when the birds are hung on the line at the processing plant. The location of the bruising is shown in Figure 6, along with other common sites for carcass bruising.

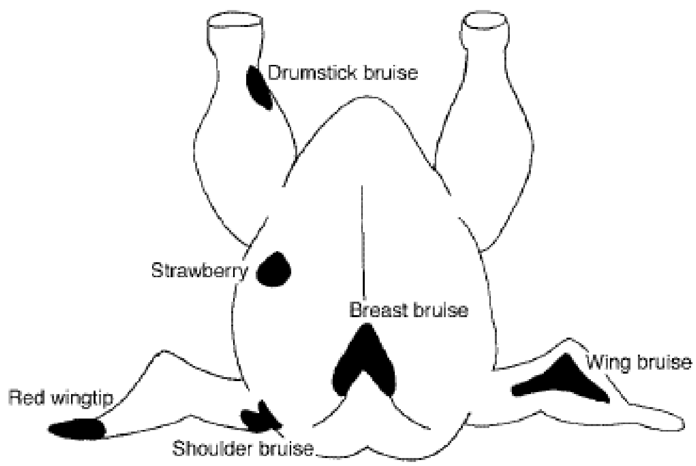


Figure 6. Common sites of bruising in poultry carcasses.

Most of the carcass bruising seen in poultry carcasses, however, occurs post-mortem as the birds are being plucked or as a consequence of the stunning process.

When bruising occurs before slaughter there is a risk that the bruise will carry *Staphylococcus aureus* bacteria. The way in which the contamination occurs is not fully understood, it is believed that proteolytic enzymes in the bruised tissue increase the permeability of the chicken skin and bruised tissue, and this allows entry and penetration of bacteria.

Birds can also acquire broken bones during pre-slaughter handling (Gregory and Wilkins, 1992). Crushed skulls can occur when plastic drawers in modules are used as containers for transporting the birds. (Figure 7). If a bird's head is sticking up when the drawer is being closed, it gets caught. Other damage can occur in the trunk of the body.

Figure 7. Damages due to incorrect placement in plastic drawers

From a welfare point of view the optimum temperature for slaughter-weight chickens that are reared in temperate climates is 22–24°C, prolonged temperatures above 38°C are dangerous. Normally the rectal temperature is 41°C but when it exceeds 42.5°C the birds begin to pant, beyond 45°C panting declines and may cease altogether, so as to conserve body water. Besides panting, the other means of losing heat are vasodilation in the shanks and comb, increasing the body surface area to encourage convective heat loss and drinking large amounts of water. By taking cold water into the crop, the base of the neck is cooled and this helps to lower the temperature of the blood in the carotid arteries which supply the brain. As birds do not have access to any water once they leave the farm, they no longer have this means of keeping their heads cool. Panting and vasodilation are the only cooling mechanisms available to crated birds, but the heat exchange through panting can be compromised if the humidity is high (greater than 70% relative humidity). At the other extreme, the lower critical temperature for chickens is about 16°C. This corresponds to the ambient temperature at which a bird will increase its rate of heat production in order to maintain body temperature.

The only obvious sign of cold stress in crated birds is feather erection. Cold stress is aggravated considerably if the birds are wet, because additional heat is lost through evaporation of the water. Besides protecting the birds from rain, it can be important to ensure that the crates are reasonably dry and in particular that they are free of ice before the birds are loaded.

It has been demonstrated that if the birds are stressed before slaughter and this causes depletion of their muscle glycogen, the meat is likely to be more tender. The greater tenderization is probably due to the high pH of the meat allowing greater enzymatic breakdown of the myofibrillar proteins in the meat fibres (Etherington *et al*., 1990).

If, on the other hand, the birds struggle and flap their wings excessively just before they are killed, this will cause a build-up of lactic acid in the breast muscle and the meat is likely to be tough. In this situation the muscle is not glycogen depleted. Instead, it becomes acidic whilst it is still hot. The relationship which reflects this effect is shown in Figure 8 (Khan and Nakamura, 1970).

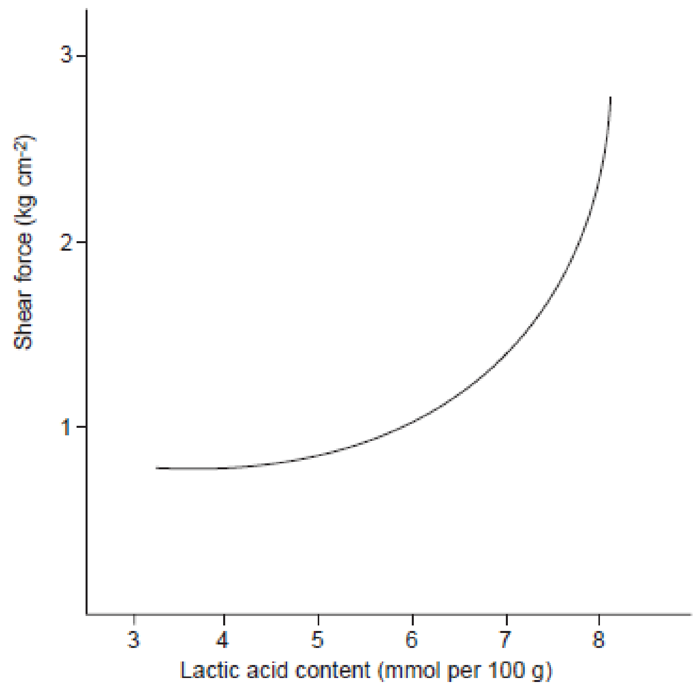


Figure 8. Relationship between lactic acid content of chicken breast muscle immediately after death and shear force after 24 h ageing.

As the lactic acid content in the muscle at the time of slaughter increases, so also does meat toughness 24 hours later. The increased toughness is due to more extensive muscle contraction during rigor development, and this effect is temperature dependent. The higher the temperature as the muscle goes into rigor, the stronger will be the contraction. This is known as heat shortening. The relationship between muscle temperature and the degree of shortening in breast and thigh meat is shown in Figure 9. Two incidental points arise from this figure. Firstly, breast meat is more prone to heat shortening than thigh meat; and secondly, broiler meat is not prone to cold shortening.

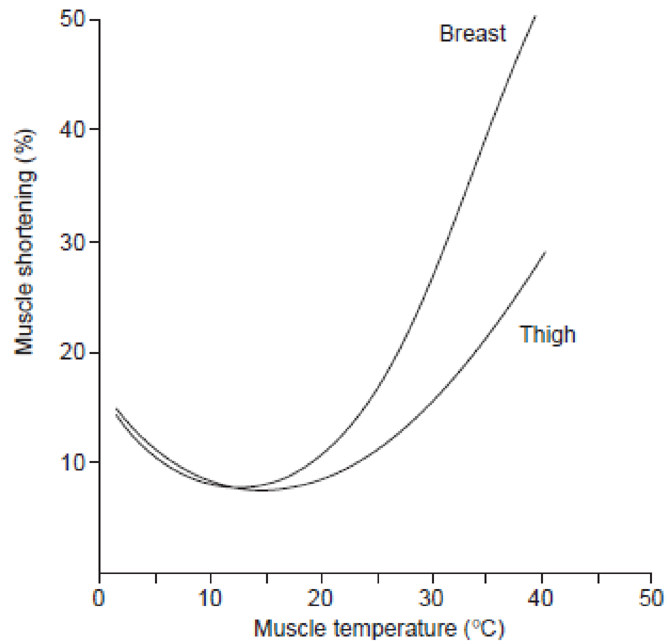


Figure 9. Relationship between muscle temperature and muscle shortening in chicken breast and thigh.

Meat toughness due to heat shortening has also been observed in broilers that have been heat stressed before slaughter, and in birds that convulsed violently as they died because they were not stunned before neck cutting. Breast meat from heat-stressed broilers is also prone to producing more drip after filleting (Northcutt *et al.,* 1994). In turkeys, it is likely to be paler as well as having a lower water-holding capacity. The paleness is due to a higher reflectance, and the meat is less red probably because the hem pigments are denatured by the high temperature–acid conditions. This type of turkey meat is similar to PSE meat in pork. In general, toughness in heat shortened poultry meat does not resolve when the meat is left to age for 48 hours.

The way birds are treated before slaughter can also influence the force required to pluck their carcasses. Normally, efficient plucking is achieved by scalding the carcass, which reduces the force required to remove the feathers, and by ensuring that the presentation of the plucking machine to the carcass allows good coverage and contact between the plucker fingers and the feathers. It is widely accepted in the poultry processing industry that the birds will be more difficult to pluck if they experience cold weather before they are slaughtered. Part of this effect could be due to cold birds entering the scalder and lowering the temperature of the water, and part of it could be due to residual activity in the sympathetic nervous system, which would cause tightening of the smooth muscle in the skin that grips the feather shaft. In practice, increasing the force of the plucker or raising the temperature of the scald-water are the most appropriate options, provided they do not damage the carcass or cook the breast meat. Excessive exercise stress before slaughter can also make feather removal harder, as can fasting the birds for longer than 8 hours.

6.2 Welfare problems of poultry at abattoir

6.2.1. Shackling

When the farmed birds are transported to slaughter plants where they are first uncrated and then, if water bath stunning is used, they are inverted and hung by their legs in metal shackles on an overhead conveyer. The conveyer moves the birds toward the electrified water-bath, where their heads make contact with the electrically charged water. When being hung, individual variation in leg diameter may cause hanging operators to use considerable force to pull thick shanks (legs) into narrow shackles (Gregory & Bell, 1987; Sparrey & Kettlewell, 1994).

In broilers there is considerable variation in the thickness of the birds' shanks, particularly between males and females. The size of the gap in the shackle where the bird's leg is held also varies (range between plants is about 0.95–1.4 cm). This means that birds with thick shanks require more force when inserting their legs into the shackles. Compression of the periosteum of the bone in this situation is potentially painful for the birds. Nociceptive properties of the skin over the legs of birds provide evidence that shackling is painful (Gentle, 1992; Gentle & Tilston, 2000). This pain is likely to be worse in birds suffering from diseases or abnormalities of leg joints or leg bones (Danbury, Weeks, Chambers, Waterman-Pearson, & Kestin, 2000) and those with dislocated joints or bone fractures induced by rough handling during catching, crating, and uncrating. The average force applied on the chicken's leg during shackling is about 90 N. A force of 75 N applied to the shank is sufficient to cause a rise in heart rate in a chicken, this kind of force is painful, and this is less than the normal shackling force.

The tightness of the shackle is important in two other respects. Firstly, the fit should be sufficiently tight to ensure good electrical contact between the shackle and the bird (when using constant voltage stunners), otherwise there is a risk of inadequate current flow. Secondly, the grip must ensure that the bird does not become dislodged from the shackle during plucking. Clearly, there is a compromise between the need to provide a low electrical resistance at the shackle electrode, thus ensuring an adequate stun, and not hurting the bird through compression of the shank. Some broiler plants and many turkey plants use shackles which have a choice of gap widths for birds of different shank thicknesses.

Birds may "struggle violently" and males, who have thicker shanks, struggle sooner and longer than females (Satterlee, Parker, Castille, Cadd, & Jones, 2000). Bruising of the surface of leg and thigh muscles often occurs (Lambooij, Pieterse, Hillebrand, & Dijksterhuis, 1999; Raj, 2004). Conflicting bird-welfare concerns involve using tightfitting shackles. Although they may provide good electrical contact between the legs and metal shackles, they are likely to increase the severity of the pain associated with shackling (Sparrey & Kettlewell, 1994). Hanging upside-down is a physiologically abnormal posture for chickens, and handling, inversion, and shackling are "traumatic" and stressful (Kannan & Mench, 1996; Kannan, Heath, Wabeck, & Mench, 1997; Debut et al., 2005; Bedanova *et al*., 2007). Therefore, it is advisable to minimize the time between shackling and stunning. For these reasons, approximately 90% of birds flap their wings immediately after shackling, and 66% flap their wings during any unevenness they experience in the line (Kannan *et al*., 1997). This is a natural response in a bird which is either losing its balance or falling. It is likely that such wing flapping could lead to dislocated joints and/or broken bones, which has not been quantified. However, when birds exhibit wing flapping while shackled, these vigorous movements can lead to bruising and hemorrhages of the wing tip that cause carcass downgrading (Gregory, Austin, & Wilkins, 1989).

6.2.2 Pre-stun Electric Shocks

It is well-documented in the scientific literature that some birds can inadvertently experience painful electric shocks before making contact with the electrified water bath (Schütt-Abraham, Wormuth, & Fessel, 1983; Gregory & Bell, 1987; Sparrey, Kettlewell, Paice, & Whetlor, 1993; EFSA, 2004b; Gazdziak, 2007). This can happen when a bird's wing makes contact with the water before the head or if wing-flapping occurs at the entrance to the stunner. Turkeys are especially prone to pre-stun shocks (Gregory, 1994; Wooton & Gregory, 1991) because their wings hang lower than their heads when hung inverted on a shackle. In some slaughter plants, pre-stun shocks may occur because the ramp at the entrance to the stun bath is electrically live (Gregory, 1994); although newer entry designs in broiler chicken stunners may prevent overflow of electrically charged water onto the entry ramp (Bilgili, 1999).

6.2.3 Stunning and slaughtering

The methods used for stunning and killing poultry can affect the following conditions in their carcasses:

- physical convulsions;
- wing and shoulder haemorrhages;
- breast skin colour and haemorrhages;
- breast meat haemorrhages;
- bleeding efficiency;
- breast meat pH fall;
- breast meat texture;
- ease of feather removal;
- removal of inedible offal;
- hygiene of the body cavity.

Table 3 summarizes the causes of downgrading for the haemorrhagic conditions. Research experience indicates, as widely described in other parts of this thesis, that breast meat haemorrhages are inevitably linked with the use of electrical stunning with a low frequency waveform. Also wing haemorrhages in broilers have a cause in the electrical water-bath, but the situation is complicated by the fact that wing haemorrhages can also be due to inadequate bleeding and unduly harsh plucking when removing the feathers.

So, red wingtips, wing haemorrhages and red feather tracts may depend on the electrical stunning, but are worse in the badly bled carcasses. As the severity of plucking increases, so also did the prevalence of red wingtips, wing haemorrhages, red feather tracts and broken wishbones. Breast meat haemorrhages within the meat are not affected by either the efficiency of bleeding or the severity of plucking.

Table 3. Causes of haemorrhaging in broiler carcasses.

Downgrading problem	Cause
Red wingtips	Excessive flapping of the wings before death Stunning process Poor bleeding in combination with harsh plucking
Wing and shoulder haemorrhages	Stunning process Poor bleeding in combination with harsh plucking
Red feather tracts and neck skin	Largely due to poor bleeding
Breast muscle haemorrhages	Usually due to high stunning currents. Not influenced by efficiency of bleeding or severity of plucking

The reason for the effects on the wings was due to the fact that when a shackled bird is stunned and bled, its wings go limp as the bird dies and they hang down from the shoulder joint. If this occurs before blood emptied from the wing veins, the veins will contain blood when the carcass enters the pluckers. The rotating plucker fingers can rupture the veins through the skin, causing a post-mortem bruise; the more severe the plucking, the more likely it is that a bruise will form. Poor bleeding from the neck-cut wound increases the likelihood of the wing veins being engorged with blood, and severe plucking increases the likelihood of wing vein rupture.

High frequency electrical stunning can reduce the prevalence of breast muscle haemorrhages (by as much as 100%) in comparison with low frequencies. Also gas stunning using a combination of 30% CO_2 and less than 2% O_2 reduces the amount of breast muscle haemorrhaging in broilers and turkeys.

Consumers often look upon broken bones in poultry portions as ‘foreign bodies’ and the bone fragments can inflict mouth damage if they have sharp edges. If the wishbone (furculum) breaks, a section of the bone may be left attached to the breast meat when it is filleted. Wishbones usually break during the initial body spasm at stunning and during plucking. Increasing the stunning current can increase the prevalence of broken wishbones.

Electrical stunning also results in broken coracoid and scapula bones, but this does not occur with gas stunning (Raj et al., 1997). Automatic neck cutters bleed the birds out by making a cut at the back of the neck.

Automatic neck cutters are sometimes set to sever the spinal cord. This makes it difficult to judge whether the bird is conscious or unconscious. By severing the spinal cord, the nervous activity passing from the brain to the body is interrupted. This helps to reduce carcass convulsions and it quickly arrests the smooth muscle activity that is responsible for vasoconstriction and for gripping the feather shafts in the skin. In birds which are not scalded, feather removal is easier if the spinal cord is severed at neck cutting (Levinger and Angel, 1977). There could also be a poorer bleed-out if the spinal cord is cut, because it would result in a prompt vasodilation before the blood has been lost.

The way birds are stunned and slaughtered affects the way in which they convulse as they die, and the convulsions affect muscle metabolism and meat quality. The general principle is that increased muscle activity results in a faster rate of pH fall. If this occurs whilst the muscle is hot, there is a greater risk of toughness due to heat shortening. If birds are bled without being electrically stunned, as in religious slaughter, they convulse violently during bleeding and this causes an accelerated rate of breast muscle pH fall. At high stunning currents the carcass quickly relaxes after it leaves the stunner, and so the rate of breast muscle pH fall is slower. If the carcass is cut into portions and filleted within 4 hours of slaughter, the freshly filleted breast will contain more ATP and so it will be more prone to toughness caused by rigor shortening (Papinaho and Fletcher, 1996). If, on the other hand, the carcass is left whole or the breast is filleted after the muscle has been allowed to metabolize its ATP (i.e. more than 4 h after slaughter), texture is either unaffected or slightly more tender in the birds which received a high stunning current. However, if the stunning current is applied for long periods (e.g. 10 seconds in comparison with 4 seconds), the longer forced contraction can accelerate glycolysis and lead to tougher breast meat through heat shortening (Young et al., 1996). One way of accelerating post-mortem glycolysis whilst minimizing heat shortening is to stun with 30% CO_2 and 60% argon (Raj et al., 1997).

During electrical stunning, some of the birds defecate and urinate. This is particularly noticeable at high stunning currents, and it causes bacterial contamination of the water in the stunner. About one-third of the birds inhale water during the initial tonic spasm that occurs during stunning, and this allows bacterial contamination of the body cavity with dirty stunner water when the respiratory tract is removed during evisceration.

Effectively stunned birds who have not undergone cardiac arrest at stunning must be killed quickly, otherwise they will regain consciousness (Gregory, 1986). The duration of unconsciousness induced by a stun depends on the amount and frequency of the current (Raj, 2003). Even if birds are adequately stunned, they may regain consciousness during bleed-out and enter the scald vat while alive if the neck-cutting step is not performed quickly or if it is ineffective. The neck cut is usually performed automatically with a machine but it can be also manual. The automatic neck cutters usually make a cut at the side or back of the neck. A ventral neck cut, which severs both carotid arteries (see Figure 10, is more effective at inducing a rapid death than a neck cut that severs only one carotid artery (Gregory & Wotton, 1986).

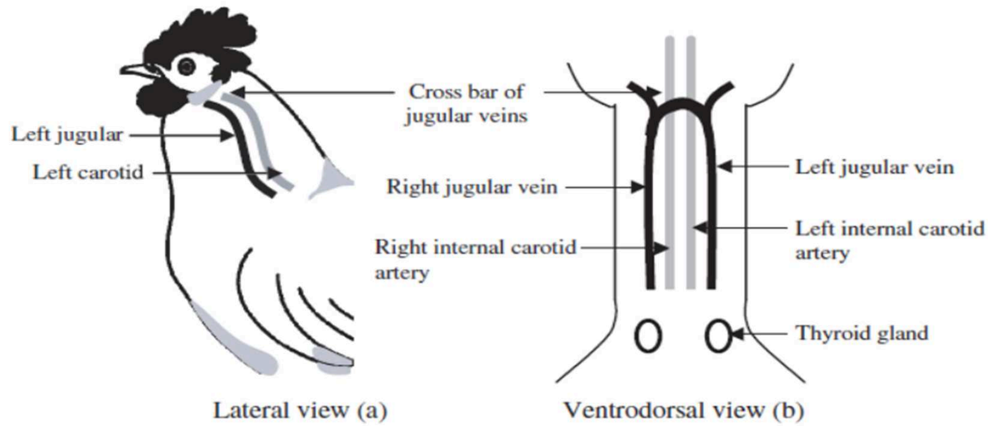


Figure 10. Blood Vessels in chicken. (a) Lateral view after skin removal: Muscle *(M. longus colli ventralis)* and Trachea (b) Ventrodorsal view after skin removal: Muscle *(M. Sternohyoideus),* Trachea and Oesophagus.

Severance of both the carotid arteries in the necks of differently sized birds may not always be possible when using neck-cutting machines, and it is not known how often birds miss the knife or blades. This because the killing machine is fixed at a specific height whereas the birds' heads may hang at different heights if there are variations in bird size.

Failure to sever the carotid arteries greatly increases the risk of resumption of consciousness during bleeding if the birds do not develop a cardiac arrest at stunning. Inducing a cardiac arrest is the most effective method of ensuring that consciousness will not return.

The optimum time between stunning and neck cutting is determined by three factors. Firstly, it should not be so long that it allows the birds to regain consciousness before they die. Secondly, it should be long enough to allow the supervisor to assess whether the birds are stunned when they leave the water bath. Thirdly, the birds should be given sufficient time to allow their necks to relax and thus feed into the guide bars of the automatic neck cutter. On average the time to neck relaxation after the end of stunning is 9 seconds in broilers, but this may be influenced by whether they experience a cardiac arrest.

It is also worth mentioning that cervical dislocation of poultry frequently leads to decapitation, and the European Council Regulation 1099/2009 stipulates that cervical dislocation “shall not be used on more than seventy animals per day” and also establishes that this method cannot be used in birds that have a live-weight over 5 kg.

6.2.4. Bleeding

Opening the blood vessels in the neck results in bleed-out.

The bleed-out phase must be very fast, but it depends on factors such as bird size and type. During the process, about 35–50% of the total blood is lost; however, considerable variation can exist between animals and flocks. Other factors affecting blood loss include stunning method used and time interval between stunning and bleeding. It is important to note that a poor bleed-out can increase the prevalence of carcass downgrading conditions due to blood spots and, in particular, engorged or haemorrhagic wing veins (Gregory, 1989; Gregory and Wilkins, 1989).

Chapter 7
Poultry processing in a commercial slaughter plant

All the experimental activity described in the present thesis has been carried out in a major Italian poultry processing plant, where usually more than 50,000 broiler chickens per day are slaughtered. In this chapter, a general description of the main operations (from the arrival of the animals to the carcass chilling) occurring at this plant is provided.

7.1 Receiving and weighing

The process starts with a bulk weighing of birds received on the truck, once the truck enters the processing plant. The live weight is used as the basis for calculating the payment to the farmer.

7.2 Unloading

After weighting, the animals are placed in a mechanically ventilated parking area waiting for their positioning on the processing line (Figure 11).

The birds are then manually unloaded from the crates and hung on the shackle line (see Figure 12).

Figure 11. Parking area

During the unloading operation, special care is taken by personal to minimize bruising and lesions to the birds.

All the handling operations before stunning are carried out exposing the birds to a special light (mainly blue) that has been demonstrated to prevent the animals from stress and excitement.

Figure 12. Unloading and shackling before stunning

Shackle line is also provided with a breast comforter (a plastic strip that is hung parallel to the line so that the birds rub against as they are conveyed to the stunner).

Figure 13. Birds hung before stunning

7.3 Stunning

Stunning operation is carried out by means of an electrical water-bath. The commercial stunner used is a LINCO® *Water Stunner BA4* (see Figure 15 and Figure 16). The stunner allows to set the electrical frequency and the voltage level. This latter is adjusted to apply, for each broiler in the water bath, a sine wave Alternating Current (AC) at a desired level. A digital display shows, other than the actual voltage and frequency applied, the total current level in the water bath.

The water bath tank allows the presence of up to fourteen birds; the number of birds simultaneously present in the water bath depends on the size and live-weight of the birds. Along the line, at the exit of the water bath, there is an operator that assesses the birds' unconsciousness following the stunning operation.

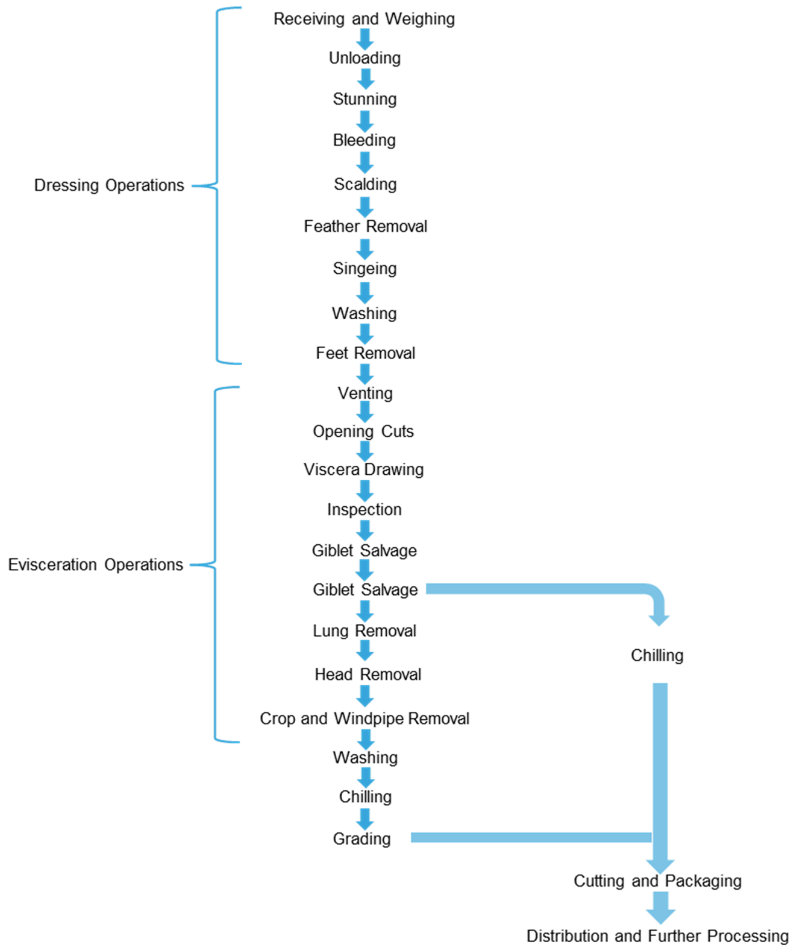


Figure 14. Schematic flow chart of the main operations at the slaughter plant

Figure 15. Electrical stunner

Figure 16. Birds at the exit of water bath stunning

7.4 Bleeding

Opening the blood vessels in the neck results in bleed-out. The broilers are slaughtered manually using a unilateral neck-cutting procedure that severs the right common carotid artery and external jugular vein (see Figures 17, 18 and 19). The blood vessels are severed just below the jowls so that head, windpipe and oesophagus remain intact.

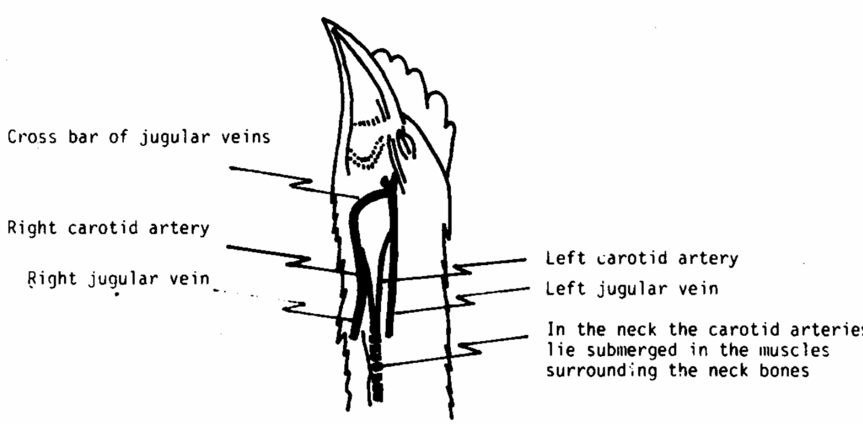


Figure 17. Neck blood vessels in poultry

Figure 18. Cut of neck blood vessels

Figure 19. Bleeding

7.5 Scalding

Scalding is the process of immersing the birds in warm water to loosen the feathers. A soft/semi-scalding is used in the present slaughter plant. This method does not damage the outer layer of the skin but still allows a relatively easy removal of the feathers. In the scalding operation, adequate agitation of the water and uniform water temperature is essential to ensure a good feather removal.

7.6 Feather removal

Feather removal is performed by means of mechanical pickers/pluckers equipped with rubber fingers that rub the feathers off the carcass (Figure 20). This operation is done while the carcass is hanging upside down and moving forward (i.e., carried by the shackle line) in between two/three sets of drums or disks covered with rubber fingers.

Figure 20. Feathers removal by means of mechanical pluckers.

After de-feathering, head and crop are removed and put in a rake for inspection.

A device first positions the heads of the suspended carcasses in a trough-like structure. This is done with a guiding rail structure and the assistance of a worm screw device that rotates inside the trough. While the carcass is moving forward, the head is pulled and breaks at the weakest point between the atlas and the axis vertebrae. The advantage of this device is that the oesophagus and trachea (windpipe) can also be pulled out gradually and removed from the carcass.

7.7 Evisceration

During this operation, the body cavity is opened and the viscera is withdrawn (Figures 21, 22 and 23). This process is fully automatic using a circular cutting blade and a scoop-like arm to withdraw the viscera.

Figure 21. Evisceration room

The viscera is withdrawn from the body cavity but remains close to the body for inspection purposes. Once the viscera pack is exposed/removed, the birds are inspected.

Figure 22. Evisceration

Figure 23. Carcasses after evisceration

7.8 Inspection

The attached or detached viscera can reveal diseases/problems with the internal organs. The inspection operation is essential to ensure that only wholesome birds, free of disease, will get to the marketplace. The inspection area has an adequate bright light, a hand-washing station, a rake for placing suspected birds and a bin for condemned birds.

The viscera is removed after inspection and giblets (liver, heart and gizzard) are salvaged and washed in a separate line. The lungs, are removed after inspection.

Showers are used at different points of processing lines after the evisceration to wash the bird (Figure 24 and Figure 25). These devices have multiple spray points that cover most of the outside part of carcasses. This system ensure removal of any debris or blood clots.

Figure 24. Shower before chilling

Figure 25. Shower before chilling

Figure 26. Carcasses during chilling

7.9 Chilling

At this stage of the processing line, the meat has to be quickly chilled to minimize microbial growth. To this aim, air chillers are used (Figure 26). Also an overhead railing system exists. This goes back and forth along the chilling tunnel; the air is blown over cooling elements and then circulated around the room at a fairly high speed.

The chilling tunnel capacity and the amount of product allow that chilling is achieved within 150 minutes from the entry of carcasses.

Advantages of using air chilling are no moisture pickup and a drier final product that does not show much exudation (drip loss) when packed in trays.

After chilling, the birds are weighed, graded and later packed or deboned for further processing in the adjacent cutting lab.

Aim of the thesis

The present thesis deals with the improvement of animal welfare at a commercial poultry slaughter plant. In this context, a first aim of the work was the optimization of the electrical stunning process thought the identification of electrical parameters that allowed an effective water bath stunning. The effect of these parameters on occurrence of haemorrhagic lesions in both carcasses and meat was also an objective of this study.

The present work also aimed to use the slaughter plant as an epidemiological lab to investigate on the incidence and nature of myopathies and other abnormalities that affect meat of broiler chickens. Among these, the incidence of Deep Pectoral Myopathy (DPM or Oregon disease), the incidence of DFD conditions and other stress-induced muscle degenerations were investigated.

Chapter 8
Experimental activity and results

8.1 Optimization of Electrical Stunning Parameters in a Multi-Bird Water Bath

The aim of the present study was the determination of an optimal combination of stunning electrical parameters (i.e. current level and electrical frequency) to ensure an effective water bath stunning to broiler chickens during the slaughter process.

8.1.1 Materials and Methods

The experimental tests have been carried out in a major Italian commercial poultry processing plant using a stunner with a water bath able to contain up to fourteen broiler chickens. The slaughter line speed was set up to ensure for each bird a minimum stunning time of 6 s.

A digital ammeter provided with a display placed on the stunner allowed to monitor the actual total current level in the water bath. As the birds simultaneously present in the water formed a parallel pathway of resistance, calculations for the average current passing through the individual birds were made dividing the total current amount measured at the water bath by the number of birds (fourteen) simultaneously submerged in the water.

A commercial stunner (*LINCO®* Water Stunner BA4) was used to apply a sine wave Alternating Current (AC) at frequencies of 200, 400, 600, 800, 1000 and 1200 Hz. Voltage was adjusted on the stunner to obtain an estimated average root mean square (RMS) current for each broiler in the water bath of 100, 150, 200 and 250 mA.

The effects of each combination of stunning electrical parameters were evaluated on 50 (consecutive on the slaughter line) commercial broiler chickens (genetic line *Ross 708*). So a total of 800 birds was considered. All the birds were six to seven week old and weighed on average 3.73 ± 0.26 kg (± standard deviation).

The broilers were slaughtered manually using a unilateral neck-cutting procedure that severed the right common carotid artery and external jugular vein.

The presence of corneal reflex (blinking response elicited by touching the cornea), the occurrence of spontaneous eye blinking and a positive response to comb pinching were used as indicators of the state of consciousness and sensibility of the birds, as result of an ineffective or poor stun. These parameters were assessed at two key stages of the slaughter line: immediately after stunning and during bleeding, until death occurred.

8.1.2 Results and Discussion

Table 4 summarizes the results of the experimental tests on broilers. In particular, the table shows, for each tested stunning treatment (i.e. for each combination of electrical frequency and RMS current level), the corresponding number of birds with a suppression of corneal reflex and the number of birds with a positive response to a painful stimulus such as the comb pinching.

The use of some combinations of stunning current and frequency produced in some birds a spontaneous blinking response that can be assessed as a physical reflex and considered a generic sign of consciousness (Prinz *et al*., 2010; Erasmus *et al*., 2010). In particular, this happened at a frequency of 800 Hz with a current of 250 mA and at a frequency of 1000 Hz with a current of 200 mA; for these values a small percentage of birds (10% and a 12%, respectively) exhibited a spontaneous blinking response.

As shown in Table 4, however, for these combinations of stunning parameters a high number of broilers presented a negative response to corneal reflex. So, the occurrence of spontaneous eye blinking can be considered in this case as a mere muscular vibration rather than an expression of consciousness (Prinz *et al.*, 2010).

Table 4. Effect of different stunning treatments on the number of birds with suppressed corneal reflex and on the number of birds with a positive response to comb pinching.

Frequency (Hz)	nº of birds	Average body weight (±SD) (Kg)	Average Estimated Current/bird (mA)	nº of birds with negative corneal reflex	nº of birds positive to comb pinching
200	80	3.5 (±0.23)	100	27	4
	50	4.2 (±0.12)	150	33	2
400	50	3.5 (±0.21)	150	32	3
	50	3.5 (±0.26)	200	34	0
	50	4.4 (±0.21)	250	40	0
600	50	3.5 (±0.32)	150	28	3
	50	3.5 (±0.28)	200	35	1
	50	3.5 (±0.30)	250	39	0
800	50	3.8 (±0.25)	150	32	4
	50	3.8 (±0.17)	200	34	1
	50	3.8 (±0.19)	250	40	0
1000	50	3.5 (±0.21)	150	19	4
	50	3.8 (±0.23)	200	30	1
	50	3.8 (±0.24)	250	32	0
1200	50	3.8 (±0.18)	200	28	2
	50	3.8 (±0.15)	250	29	1

Statistical analysis showed that the electrical stunning frequency (Hz) had a significant effect (P=0.0004) on the occurrence of animals with suppressed corneal reflex. The effect of the stunning RMS current (mA) and the effect of the interaction of stunning frequency and stunning current were also significant ($P < 0.0001$). On the contrary, the average live body weight of the broilers did not show any significant effect on the occurrence of birds without corneal reflex (P =0 .14).

The stunning frequency was not significant for the number of birds with a positive response to comb pinching ($P = 0.5$), whereas the stunning current level showed a significant effect on the number of birds with a positive response to this painful stimulus ($P < 0.0001$).

In the model shown below and obtained by a least square regression on the number of animal without corneal reflex after the stunning treatment, P is the estimated percentage of broilers with a suppressed corneal reflex that was modelled as a function of the stunning frequency and stunning current; the standard error in the parameter estimates is shown within brackets.

*P =41.26 (6.04) -0.023 (0.0048)*Frequency (Hz) + 0.202 (0.033)*Current (mA).*

The model fitted the experimental data with a Root Mean Square Error (*RMSE*) of 5% and a coefficient of multiple determination (*R-Square* value) of 0.8.

In Figure 27, this estimated percentage of birds with negative corneal reflex is plotted against stunning frequency (Hz) for different levels of stunning current (mA)

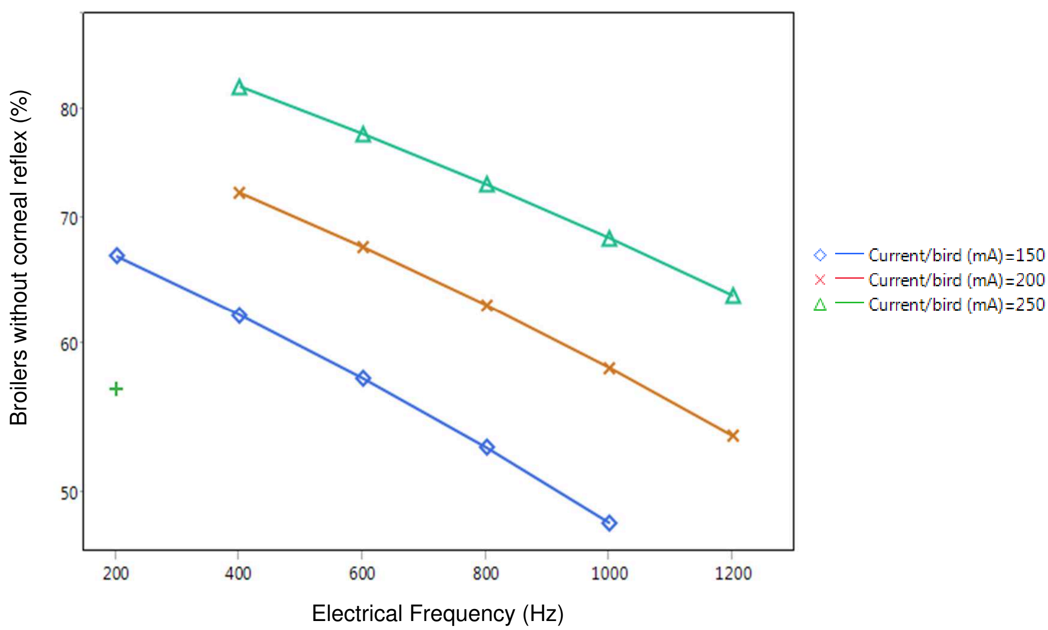


Figure 27. Estimated percentage of broilers with negative corneal reflex (%) as a function of electrical frequency (Hz) and stunning current level (mA)

The graph clearly shows that an increase in the electrical stunning frequency at a chosen RMS current level produces a decreasing number of birds with suppressed corneal reflex. Moreover, an increase of the amount of RMS current with a chosen frequency produces an increased number of broilers without corneal reflex. So, as a consequence, when the stunning frequency is increased, a higher amount of current is necessary to achieve an adequate number of birds without corneal reflex.

As widely described in literature and in previous chapters of this thesis, the absence of corneal reflex is considered a reliable indicator of deep unconsciousness and insensibility in poultry (Prinz *et al*., 2010; Erasmus *et al*., 2010; EFSA, 2013). A positive response itself, however, does not necessarily indicates sensitivity in broilers and ability to perceive pain. In fact, it is well known that the corneal reflex is a brain stem reflex and can be elicited in birds even under deep anaesthesia. For this reason, it can be expected that a limited number of animals might still show a positive response for a short period following the stunning. Prinz *et al*. (2010, 2012) suggested that under practical field conditions *a minimum of 65-70% of broilers with a suppressed corneal reflex* can be used as an indicator to identify an acceptable stunning. Studies based on the comparison between EEG results and the occurrence of corneal reflex confirmed this assumption.

If this indication is assumed, from the analysis of the graph in Figure 27 it appears clear that the probability of inducing an effective stunning with a given current is limited to a narrow range of electrical frequencies. RMS current levels of 150 mA, for example, can be used for an acceptable stunning (with more than 65% of birds with a negative corneal reflex) only if frequencies up to about 300 Hz are applied. Similarly, effective stunning can be achieved with a minimum current of 200 mA and 250 mA only if they are delivered using frequencies up to 800 and 1200 Hz, respectively. These findings correspond with those of Raj *et al*. (2006).

On the basis of these considerations, it was possible to transfer the data in Table 4 into a binomial scale. Combinations of stunning electrical parameters able to induce a suppression of the corneal reflex in more than 65% of birds were considered as “effective”, whereas the remaining combinations of frequencies and RMS current levels were considered as “not effective”.

A nominal logistic regression of these data allowed to calculate the probability to obtain an “effective” stunning as function of the applied electrical frequency for different RMS current levels. The related plot is shown in Figure 28.

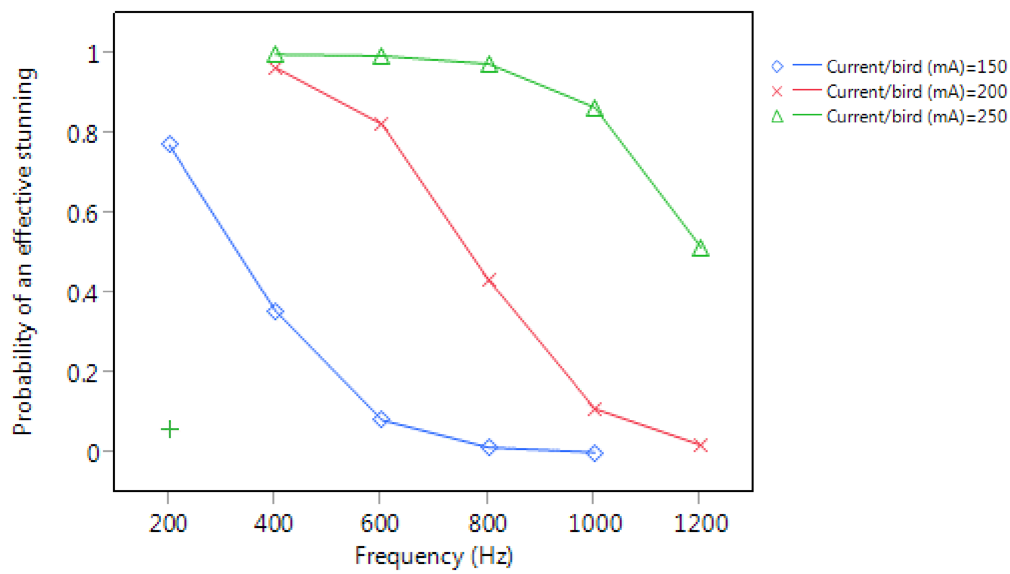


Figure 28. Probability of an effective stunning as a function of electrical frequency (Hz) and RMS current level (mA).

Also this graph confirms that the probability of an effective stunning decreases as the frequency is increased at each of the RMS current levels tested. The graph also suggests that the maximum probability of an effective stunning is obtained by using a current level of 200 mA at 400 Hz or 250 mA at 400, 600 and 800 Hz. As shown in Table 4, these combinations of stunning electrical parameters were also those that produced the lower number of birds with a positive response to comb pinching.

8.2. Effect of electrical stunning on carcass and meat quality

The present study was aimed to find the influence of the electrical stunning process on both carcass and meat quality.

In particular, a macroscopic characterization of the lesions produced by the stunning process is provided here. The effect of different combinations of electrical stunning parameters (i.e. frequency and current levels) on the occurrence of lesions on carcasses and meat was also investigated.

8.2.1. Materials and methods

A stunner with a water bath able to contain up to fourteen broiler chickens was used for the present study. The slaughter line speed was set up to ensure for each bird a minimum stunning time of 6 s. A digital ammeter provided with a display placed on the stunner allowed the monitoring of the actual total current in the water bath and the calculation of the average current passing through the individual birds.

As in the study described in the previous paragraph, a commercial stunner (*LINCO*® Water Stunner BA4) was used to apply a sine wave Alternating Current (AC) at frequencies of 200, 400, 600, 800, 1000 and 1200 Hz. Voltage was adjusted on the stunner to obtain, also in this case, an estimated average root mean square (RMS) current for each broiler in the water bath of 100, 150, 200 and 250 mA.

The effect of each combination of stunning electrical parameters on the birds carcass quality was evaluated on 50 (consecutive in the slaughter line) commercial broiler chickens (genetic line *Ross 708*). So, a total of 750 birds was considered. All the birds were six to seven week old and weighed on average 3.73 ± 0.26 kg (± standard deviation).

The birds were slaughtered manually using a unilateral neck-cutting procedure that severed the right common carotid artery and external jugular vein.

All birds were scalded and mechanically plucked (45 sec). The birds to be assessed for carcass quality were eviscerated, weighed, and held overnight at 5°C before they were examined to determine the prevalence of downgrading conditions.

The carcasses were inspected for the occurrence of haemorrhages mainly in breast muscles and leg muscles.

Haemorrhages in breast muscles were assessed both left and right *Pectoralis major* and *Pectoralis minor* muscles, whereas the *Sartorius* muscle, as well as the *Quadriceps femoris* and the *Adductor* muscles were inspected to evaluate the occurrence of haemorrhages in leg muscles.

The external appearance of these muscles and the occurrence of haemorrhages were assessed by using a 0, 1, 2 subjective scoring scale. An increasing corresponded to a worsening of the condition. So, a score of 0 indicated that the carcasses were acceptable. A score of 1 indicated that the carcass would have been downgraded because of the defect. A score of 2 indicated a defect of sufficient severity to make that part of the carcass unsuitable as a carcass or portion for the fresh or frozen trade.

8.2.2. Results and discussion

The applied combinations of stunning electrical parameters produced in the pectoral muscles haemorrhages that mainly consisted in *petechiae* (small pin-point like blood spots) and in haemorrhages with a typical oblong, striated, or branched appearance. These latter lesions were probably due to extravasating blood that followed the direction of the muscle fibres and that gave the typical striated aspect to these lesions. Similar lesions are reported by Kranen *et al*. (2010).

Frequent was also the finding in pectoral muscles of evident haemorrhages with a diameter of several centimetres (*sugillations*) near the sternum apex that extended over the connection (*raphe*) of the two superficial pectoral muscles (*Pectoralis superficialis*).

As a consequence of the stunning process, numerous petechial haemorrhages were also observed in leg and thigh muscles. These were present both in the muscle itself and in the connective or fat tissue that surrounded the muscles.

Photographs with examples of the haemorrhagic lesions found in breast and leg muscles are presented in figures 29 and 30.

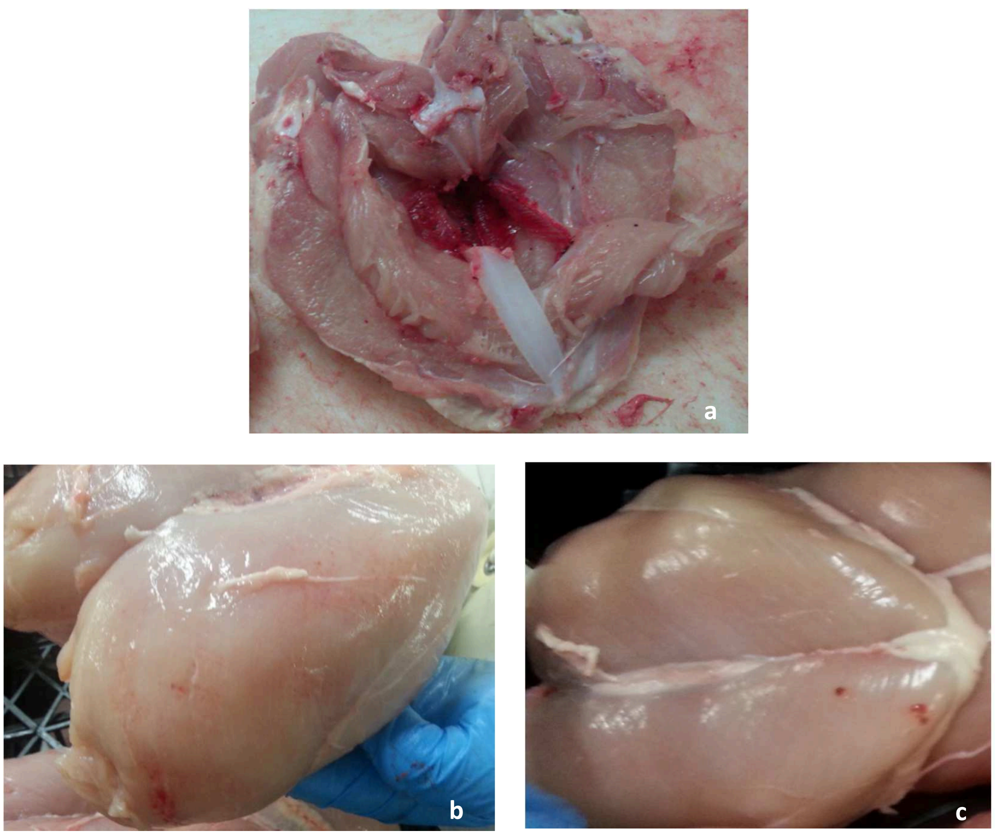


Figure 29. Different types of hemorrhages found in pectoral muscles as a consequence of the stunning process. *Figure 29a*. *Sugillation* that extended over both *Pectoralis profundis* muscles. *Figure 29b*. *Striae* and *petechiae* on the right pectoral muscle. *Figure 29c*. *Ecchymosis* on the distal side of the right pectoral muscle.

In Figure 29, different types of haemorrhages found on breast muscles are shown. A clear *sugillation*, close to the site where the breast muscle pair is attached to the apex of the sternum is shown in Figure 29a. The *sugillation* extended over both *Pectoralis profundis* muscles on either side of the connective tissue connection (raphe). Figure 29b shows the presence on the right pectoral muscle of both *striae* (where blood follows the direction of the muscle fibres) and *petechiae*, whereas three ecchymosis (haemorrhages with a surface area of several square millimetres), located on the distal side of the right pectoral muscle are shown in Figure 29c.

Figure 30a shows numerous petechial haemorrhages and *ecchymosis* in the leg muscles and in the fat and connective tissues surrounding the muscles. Petechial haemorrhages are also shown in Figures 30b and 30c, where they can be observed in the muscles of the left leg thigh.

Figure 30. Different types of hemorrhages found on leg muscles as a consequence of the stunning process. *Figure 30a. Ecchymosis* and *striae* on leg muscles and fat tissue. *Figure 30b* and *Figure 30c.* Petechial hemorrhages on the muscles of the left leg thigh.

Independently from the nature and position of these haemorrhages, they all are attributable to the electrical stunning treatment.

A soon as the electrical field is applied and resistance of the animal is surmounted, all excitable tissues are stimulated simultaneously. All muscles and muscle fibres start to contract tonically, as long as the animal is exposed to the electrical field. During this period antagonist muscles exerts severe stain each other and on the skeleton. The strongest muscles shorten upon contraction, forcefully lengthening their weaker antagonists. Tension is highest in these latter muscles and they are likely to be the main target of muscle tissue and blood vessel damage.

The rise of the intramuscular pressure generated by the muscle contraction drives the blood out of the *fasciculae* into the capacitance vessels, i.e. the post-capillary venules and small veins. Together with simultaneously induced vasocontraction of the muscular veins, these cause a sudden rise in blood pressure in the capacitance vessels. Vascular rhexis and haemorrhages are often the result.

The effect of the stunning treatments (i.e. combinations of stunning currents and frequencies) on the occurrence of haemorrhages in breast and leg muscles is shown in Table 5. For the sake of simplicity, quality defects that scored 1 and 2 have been grouped.

The table also reports the number and the average live weight (with standard deviations) of the chicken broilers used for each stunning treatment.

In the graph in Figure 31, the total proportion of breast and leg muscles with haemorrhagic lesions has been plot as a function of the electrical frequency for different RMS current levels. The graph shows a strong dependence of the occurrence of haemorrhages on electrical stunning frequency. In particular, as shown in the figure 31, the proportion of muscular haemorrhagic lesions decreased as the stunning frequency was increased.

Table 5. Effect of stunning treatments on the proportion of broilers with quality defects on breast and leg muscles.

Frequency (Hz)	nº of birds	Average body weight (±SD) (Kg)	Current/bird (mA)	Proportion of broilers with haemorrhages on breast muscles (%)	Proportion of broilers with haemorrhages on leg muscles (%)
200	50	3.5 (±0.23)	100	14	16
	50	4.2 (±0.12)	150	16	14
400	50	3.5 (±0.21)	150	6	10
	50	3.5 (±0.26)	200	2	16
600	50	3.5 (±0.32)	150	6	4
	50	3.5 (±0.28)	200	0	8
	50	3.5 (±0.30)	250	6	12
800	50	3.8 (±0.25)	150	0	6
	50	3.8 (±0.17)	200	2	6
	50	3.8 (±0.19)	250	4	12
1000	50	3.5 (±0.21)	150	2	4
	50	3.8 (±0.23)	200	2	4
	50	3.8 (±0.24)	250	2	4
1200	50	3.8 (±0.18)	200	0	0
	50	3.8 (±0.15)	250	2	4

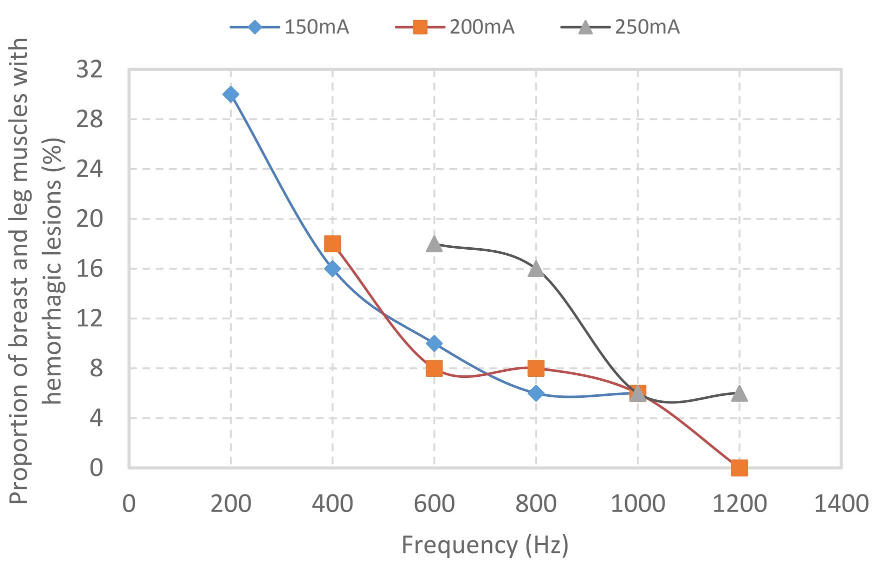
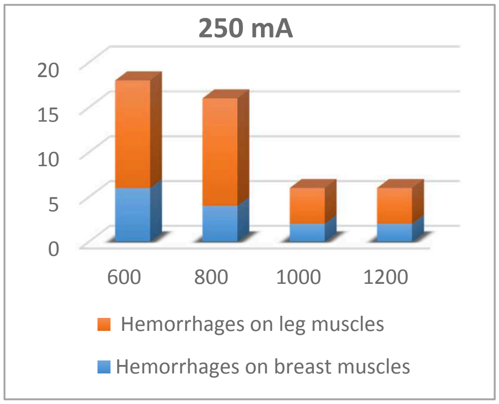
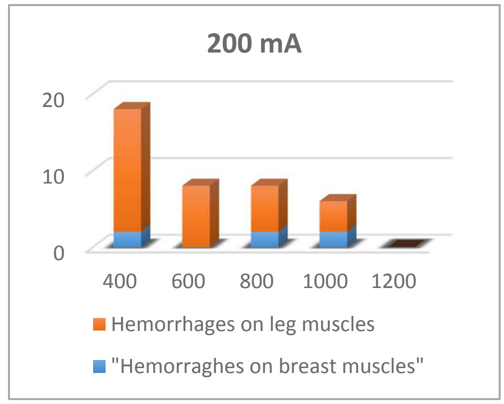


Figure 31. Occurrence of lesions in breast and leg muscles as a function of the stunning frequency for different RMS current levels

The results of statistical analysis confirmed this behaviour. A statistical significant effect of the stunning frequency on the occurrence of haemorrhages in both breast muscles (P = 0.0015) and leg muscles ($P < 0.0001$) was observed. On the contrary, the stunning RMS current level revealed to be not significant for the occurrence of haemorrhagic lesions in leg muscles ($P = 0.45$) and close to the level of significance ($P = 0.045$) for the occurrence of lesions in breast muscles.

The effect of the interaction of stunning frequency and stunning current was statistically significant for the haemorrhages in leg muscles ($P < 0.0001$) and in breast muscles (P = 0.007). The decrease in the prevalence of muscle haemorrhages as a consequence of an increase of electrical frequency is probably the result of less intensive muscle contractions during current flow, since these contractions, as mentioned before, are thought to be the cause of the rupture of the small blood vessels that is in turn responsible for the muscle haemorrhages. The influence of the stunning electrical frequency on the improvement of breast meat quality has been already reported in literature (Gregory *et al*, 1995, 1999), but, up to now, this behaviour was demonstrated only at RMS current levels lower than those tested in the present work. Our research, then, allowed to confirm the effect of electrical frequency on the occurrence of breast and leg muscle haemorrhages for RMS current levels up to 250 mA. Both the statistical analysis and the graph in Figure 31 show that, for some values of electrical frequency, there was a lack of effect of stunning current on the occurrence of muscle haemorrhages. In particular, this happened at 400 and 600 Hz for the haemorrhages in breast muscles. This behaviour is in contrast with earlier findings (Gregory et al., 1989; Wilkins *et al*., 1999, 2002), where the stunning current levels showed a significant effect on the occurrence of haemorrhages in pectoral muscles. In these works, however, frequency values lower than those used in the present study were employed.





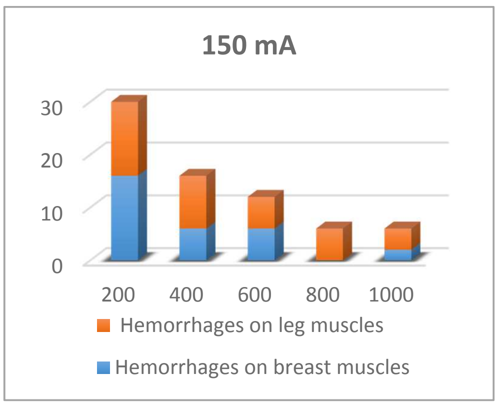


Figure 32. Relative distribution of the hemorrhages in leg and breast muscles as a function of the frequency for different RMS current levels.

From data in Table 5, it can be calculated that, globally, the haemorrhagic lesions were more frequent in leg muscles (65%) than in breast muscles (35%).

Figure 32 shows the relative distribution of the haemorrhages in leg and breast muscles as a function of the frequency for different RMS current levels. For low stunning frequency values (up to 600 Hz) and for low RMS current levels (up to 150 mA), the haemorrhages were almost equally distributed in leg and breast muscles. For higher current levels and higher frequency values the haemorrhages in leg muscles became prevalent.

In Figure 33, the occurrence of haemorrhagic lesions in only leg muscles has been plot as a function of the stunning electrical frequency for different RMS current levels.

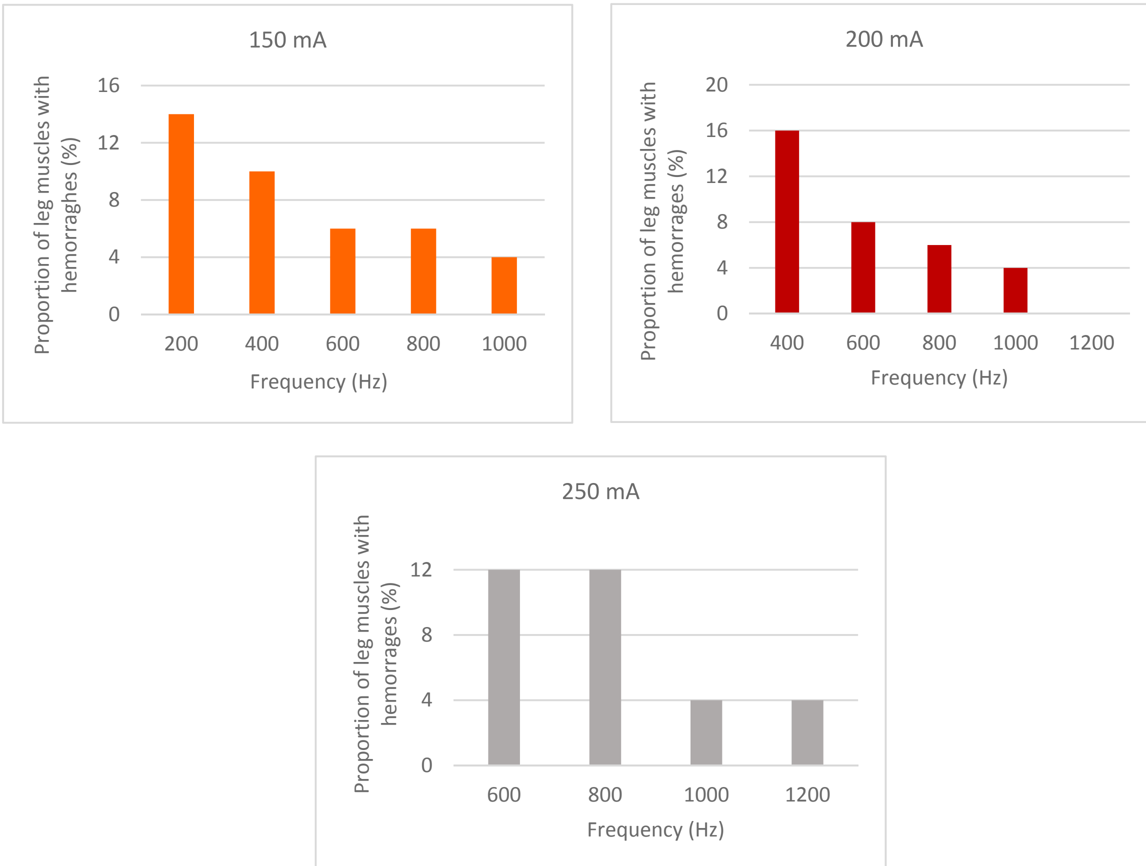


Figure 33. Occurrence of lesions in leg muscles as a function of the stunning frequency for different RMS current levels.

The figure clearly shows the strong influence of the stunning frequency on the occurrence of haemorrhages in leg muscles. In particular, an increase in the stunning frequency allowed a significant decrease in the occurrence of leg muscles with haemorrhagic lesions. The reduction was up to 70% at 150 mA, up to 100% at 200 mA and up to 66% at 250 mA. Similar results were obtained for the haemorrhages in breast muscles (see Figure 34), where an increase in the stunning frequency resulted in an overall reduction of their occurrence of up to 100%.

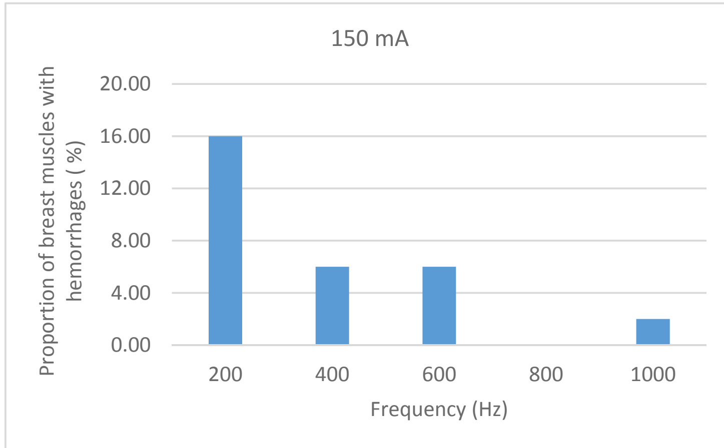


Figure 34. Occurrence of lesions in breast muscles as a function of the stunning frequency for a RMS current level of 150 mA.

8.3 Animal Welfare and Meat Quality using Electrical Water Bath Stunning

In a previous part of the present thesis, it was demonstrated that the number of unconscious birds decreased as the stunning frequency was increased for a given RMS current level. Moreover, it was concluded that the maximum probability to achieve an effective stunning could be obtained by using a current level of 200 mA at 400 Hz or 250 mA at 400, 600 and 800 Hz. However, a comparison with the data presented in the previous paragraph, shows that these combinations of stunning electrical parameters produced a high number of animals with lesions on carcasses. This demonstrates that the searching for a compromise between animal welfare and carcass quality is a very difficult task when electrical water bath stunning is used and that the achievement of an acceptable level of welfare rarely allows to obtain carcasses completely free of defects.

8.4 Meat Quality Abnormalities: Incidence of Deep Pectoral Myopathy

Deep Pectoral Myopathy (DPM) or *Oregon Green Muscle Disease* is a condition characterized by focal necrosis of the *Pectoralis minor* (*Supracoracoid*) muscles (Dickinson *et al*., 1968; Harper *et al*., 1971).

In an *early developing stage*, the entire *Supracoracoid* muscle appears pale and swollen and covered by a fibrinuous, sometimes haemorrhagic, membrane. Lesions are usually limited to the middle portion of the muscle and are often bilateral. In a *late stage*, the affected tissues become pale-grey, yellow or green in colour.

The aetiology of the disease has not been entirely recognized. It is known that the occurrence of the necrosis has a strict relation with the specific location of the *Pectoralis minor* muscle (that is surrounded by an inelastic *fascia* and the sternum), which do not allow the muscle mass to expand in response to physical activity. During normal muscle exercise, such as wing flapping, the muscle increases in weight by about 20% (Siller *et al*., 1985) or even 25%. This increase results in an elevated pressure within the muscle, which restricts blood flow and causes ischemic necrosis.

It has to be underlined that the green colour does not result from inflammation and the changes are only the result of the ischemic necrosis (Harper *et al*., 1983; Siller, 1985).The green colour probably originates from the transformation of myoglobin under anaerobic conditions.

No public health significance is associated to DPM, but it is aesthetically undesirable. The fillet should be removed, whereas the rest of the carcass is still fit for human consumption (Jordan and Pattison, 1998). However, the required trimming operations determine the downgrading of the products and produce an economic loss for the industry, especially because it affects the more valuable part of the carcass (Bianchi *et al*., 2008).

According to Siller (1985), DPM occurs exclusively in birds that have been specially selected for breast muscle development. This also because commercially reared broilers and turkeys are relatively inactive during the growing period. So, the *Pectoralis minor* muscle is not exercised and this determines a further reduction of the elasticity of the muscle compartment that does not allow the accommodation of the swollen muscle after physical exercise.

The incidence of DPM increases in higher-yielding strains and in males. So, it can only be expected to increase in the future, as selection for breast meat yield continues and broilers are reared to heavier market weights.

In the context of the present thesis, the incidence of DPM in broiler chickens slaughtered at a commercial slaughter plant has been assessed.

8.4.1. Material and Methods

The study has been carried out in an Italian commercial processing plant on a total of 37650 broiler chickens chosen randomly from 32 flocks for a period of 7 months (Sep. 2014 - Mar.2015). The broilers belonged to two commercial breeds (*Ross 708 and Ross 308)*, were reared under intensive conditions and slaughtered from 45 to 52 days of age. The average live body weight of the broilers belonging to the breed *Ross 708* was 3.75 ± 0.28 kg (± standard deviation); whereas the broilers of the breed *Ross 308* weighed on average 3.92 ± 0.32 Kg.

For each flock, an average of 1170 whole breasts were randomly selected after the breast-deboning operation in the processing line and used to establish the occurrence of DPM through macroscopic visual inspection. On the basis of the developing stage of the disease, muscles with lesions at an *early stage* (with hyperaemic and haemorrhagic appearance) were differentiated from those having lesions at a *later stage* (with a pale-grey or yellow- green colour).

The data were analysed by descriptive statistics (mean, standard deviation, minimum and maximum values). To study the influence of external factors (e.g. seasonal factors) on the occurrence of the disease the software JMP v11.2 (SAS Institute) was used.

8.4.2 Results and discussion

Figure 35 shows the typical aspect of the DPM lesions found on the *Pectoralis minor* muscle. These were classified as lesions at an *early stage* (Figure 35a) or *later stage* (Figure 35b).

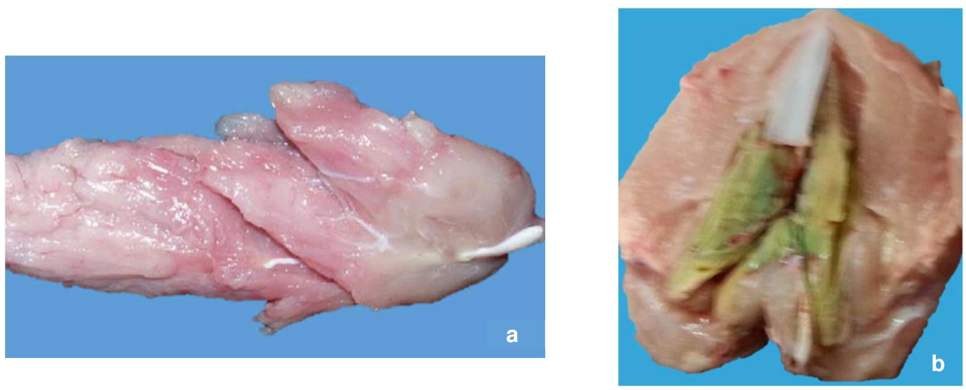


Figure 35. Deep pectoral myopathy in its early *(Figure 35a)* and later *(Figure35b)* developing stages.

As clearly shown in the figure, the early lesion was characterized by oedema, hyperaemia and discoloration of the *Pectoralis minor* muscle. Moreover, the lesion was generally sharply confined from the unaffected part of the muscle. At a later stage, the whole muscle had an intensive yellow-green colour.

In Table 6, the incidence of DPM for all the inspected broiler breasts is reported.

The table shows that the total incidence of the myopathy was 4.17%, with a minimum incidence value of 0.94% and a maximum of 9.83%. The table also indicates that the lesions at an early developing stage were more frequent than those at a later stage, with an average incidence of 2.76% and 1.41%, respectively.

Table 6. Overall incidence of DPM and percentage of lesions at early and later developing stage

	Early Lesions (%)	Later Lesions (%)	Total (%)
Mean	2.76	1.41	**4.17**
SE	0.25	0.13	**0.40**
Min - Max	0.6 - 6	0.3 - 4.2	**0.94 - 9.83**

The statistical analysis did not show any significant effect of the live body weight of the birds on the occurrence of DPM (P = 0.94). This result is in contrast with that of an early study of Bilgili *et al*., (2000) that found a dependence of the incidence of DPM on the broiler live weight, with a higher incidence in birds weighing more than 3.6 Kg. It has to be pointed out, however, that almost all broilers monitored in the present study had a live weight higher than 3.6 Kg, so a comparison with lighter broiler chickens was not possible. It has also to be considered that it is difficult to compare the results from Bilgili *et al*., (2000) obtained under experimental conditions, with those obtained in this study, which was conducted under commercial conditions.

On the other hand, the high values of body weight could be also one of the reasons of the higher total incidence of DPM found in the present study compared with that estimated by Bianchi *et al.*, (2006) to be 0.84%.

A significant effect of seasonal factors on the occurrence of the disease was also found (P = 0.02), with a higher incidence during the cooler months. This is in line with earlier findings (Bilgili *et al*., 2000, 2002). The behaviour of the average monthly incidence of DPM is shown in the graph in Figure 36.

Results of the comparison between the incidences of DPM in broilers belonging to the genetic lines *Ross 708* and *Ross 508* are shown in Table 7 and in Figure 37.

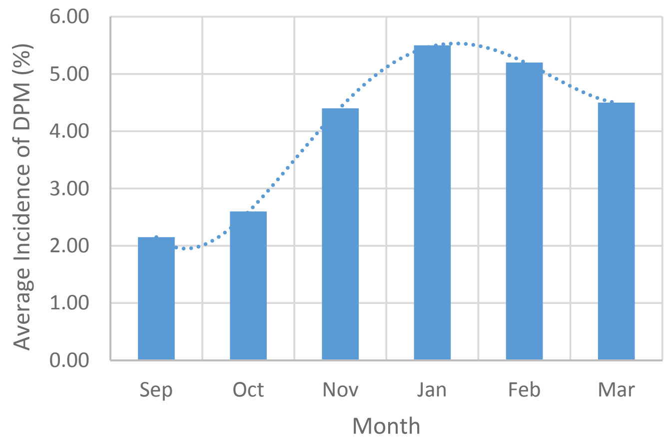


Figure 36. Average monthly incidence of DPM. The incidence was higher during the cooler months.

Table 7. Incidence of DPM in broilers *Ross 708* and *Ross 308*

	DPM incidence in broilers *Ross 708* (%)	DPM incidence in broilers *Ross 308* (%)
Mean	**3.64**	**4.75**
SE	0.56	0.55
Min - Max	0.94 – 9.83	2.38 – 8.83

As indicated in the table and in Figure 37, *Ross 308* exhibited a higher overall incidence of DPM compared to *Ross 708* (4.75% vs 3.64%); whereas *Ross 708* presented a higher variation in the incidence (from a minimum of 0.94% to a maximum of 9.83%).

In 80% of the flocks with broilers *Ross 308* the incidence was higher than 3%; the same percentages were found in only 50% of the flocks with broilers *Ross 708.*

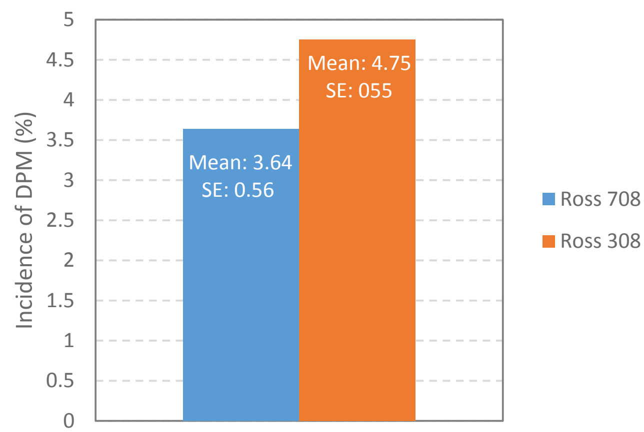


Figure 37. Comparison between the incidence of DPM in broilers *Ross 708* and *Ross 308.*

All these results clearly suggest the important role played by the genetics in the determination of DPM, even if it is difficult to explain the reason of these differences as both genotypes were commercial strains selected for breast muscle hypertrophy and fast growing.

8.4 Meat Quality Abnormalities: An idiopatic myopathy

As already described in other parts of this thesis, artificial genetic selection for improved body weight gains and food conversion has resulted in rapid muscle growth in commercial broiler chickens. Commercial strains of meat type poultry appear to exhibit an increased incidence of idiopathic and stress induced myopathies compared to their slower growing counterparts or genetic predecessors (Mitchell, 1999). This because genetic selection has resulted in profound changes in muscle fibres and vascular structure of poultry skeletal muscle (Wilson *et al*, 1990; Remignon *et al*., 2000). These alterations have led to an increasing incidence of muscular problems such as leg weakness and oedema, focal myopathy, deep pectoral myopathy and muscular dystrophy in broilers and turkeys (Sosnicki and Wilson, 1991).

The patho-physiological mechanisms of such myopathies and other muscle abnormalities have been the subject of an extensive review (Mitchell, 1999).

It is hypothesized that the increased occurrence of muscle abnormalities may be attributable to tissue growth ultimately exceeding metabolic, physiological or anatomical limits (Mitchell, 1999), although such mechanisms have yet to be fully characterized.

It is evident that, in order to support the increased growth rate of the "demand organs" such as muscle, bone, fat, skin and feathers, appropriate adaptations must occur in the "supply organs" including the cardiovascular and respiratory systems, intestine and liver. It may be postulated that failure of the supply systems to meet the demands of a disproportionately large growth rate may underlie many of the current pathologies and welfare problems encountered in commercial broiler production. Disseminated muscle fibre degenerations and hyper-contractions are particularly prevalent in the breast muscle of these broiler chickens.

A common feature of all myopathic and dystrophic conditions is the leakage of the intracellular muscle enzyme creatine kinase (CK). Thus increased plasma activity of CK is a useful diagnostic indicator of muscle pathology and altered sarcolemmal integrity (Mitchell, 1999). Myopathy as evidenced by elevated plasma CK, is associated with demonstrable histo-pathological changes in muscle tissue. The condition is characterized by histological changes indicative of muscle degeneration including hyaline (hypercontracted) fibres, fatty infiltration, fragmentation of the sarcoplasm, mononucleocyte infiltration and focal necrosis. Indicators of tissue regeneration such as basophilic fibres and internalized nuclei have also been observed (Mahon, 1999). The onset of pathological changes appears to correlate with the attainment of a specific fibre diameter regardless of age or body weight suggesting a limit for fibre hypertrophy beyond which muscle function may be compromised (Mills *et al*., 2000).

A frequent finding at the cut lab of the slaughter plant where the experimental activity of the present thesis has been carried out was the occurrence of a myopathy, whose features cannot be easily attributed to other muscle degenerations described in literature. This degeneration, which can be considered as an idiopathic and likely stress induced myopathy, had serious consequences for the meat processor as it often involved the downgrading of the affected parts with a significant economic loss.

The macroscopic and histological characterization of this myopathy and the assessment of its incidence is the object of the following part of the present thesis.

8.5.1 Material and Methods

The study has been carried on a total of 37650 broiler chickens chosen randomly from 32 flocks. The broilers belonged to two commercial breeds (*Ross 708 and Ross 308)*, were reared under intensive conditions and were slaughtered from 45 to 52 days of age. The average live body weight of the broilers belonging to the breed *Ross 708* was 3.75 ± 0.28 kg (± standard deviation), whereas the broilers of the breed *Ross 308* weighed on average 3.92 ± 0.32 Kg.

The occurrence of the myopathy was assessed both on pectoral (P) and quadriceps (Q) muscles and pH values were measured 15 min, 5 h and 24 h post-mortem on the affected parts by means of a *HANNA® Instruments (USA) pH-211 Microprocessor* pH meter.

For histological analysis, samples were frozen in iso-pentane pre-cooled in liquid nitrogen and stored at -80° C. Then, 10 µm thick cryostat sections were obtained and stained with hematoxylin and eosin (H&E) Engel Trichrome (ET) for basic morphological assessment.

To investigate the incidence of myopathy, data were analysed by descriptive statistics (mean, standard deviation, minimum and maximum values).

8.5.2 Results and Discussion

Figure 38 shows the macroscopic appearance of some breasts affected by the investigated myopathy. The pectoral muscles had a pale-pink colour and appeared sticky when touched. A light finger pressure was able to produce a persistent fovea, whereas a heavier pressure produced a breast muscle damage with rupture of muscular fibres. Similar lesions were also found on leg muscles.

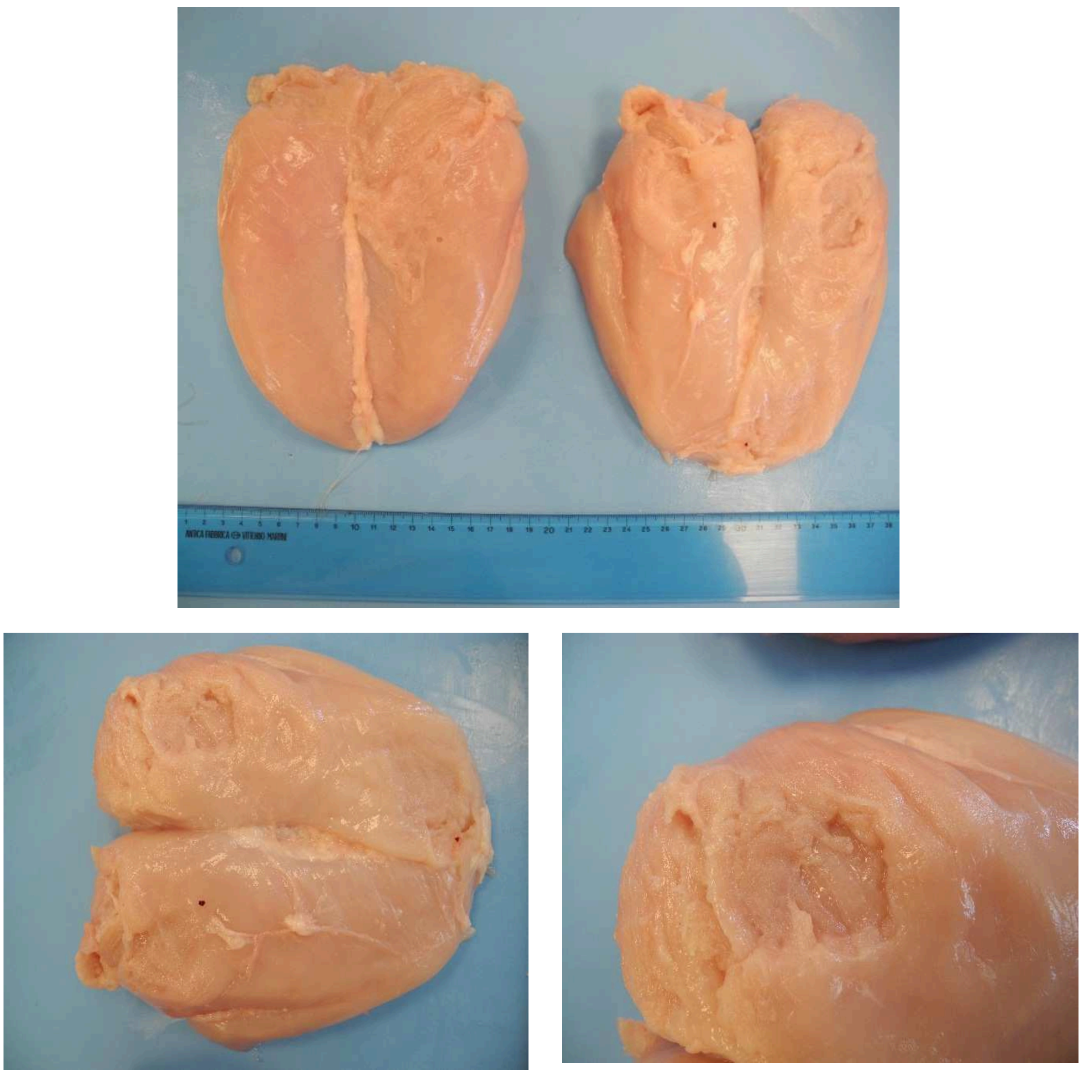


Figure 38. Myopathy of breast muscles. The muscles appeared pale-pink in colour and with a flaccid texture.

Some features of this myopathy, such as the pale colour of the muscle and its soft and flaccid texture, were similar to those of PSE-like meats. The pH values found here, however, were higher than those typically measured for PSE-like breast meats as they did not differ significantly from the PH values of the normal broiler chicken muscles. In the chicken, normal pH values at 15 min post-mortem are generally around 6.2 to 6.5, whereas normal ultimate pH values are around 5.8 (Fletcher, 1999). The degenerated muscles considered in the present study showed pH values similar to those of normal muscles: average values at 15 min post-mortem were around 6.3, average pH values at 5h post-mortem were around 6.1 and average pH values at 24 h post-mortem were around 5.9. This demonstrated that the degeneration could not be considered as a PSE-like myopathy, where ultimate pH values are generally lower than 5.7.

Histopathological findings at morphological stains (H&E and ET) showed presence of increased variability in fibre size, presence of degenerated fibres with invasion of macrophages and inflammatory cells and fibres with vacuoles. Angular atrophy and necrosis associated with sarcoclastosis was the most prominent finding in pectoral muscles. In some cases, fatty infiltration and hypercontraction of muscle fibres were also found (Figure 39 and Figure 40).

Histological analysis revealed changes that are suggestive of a generic muscle degeneration. However, histology did not give any additional information on the origin and the nature of this myopathy that can be generically classified as an *idiopathic myopathy*. An important role was certainly played by the artificial genetic selection for growth rate of the breeds

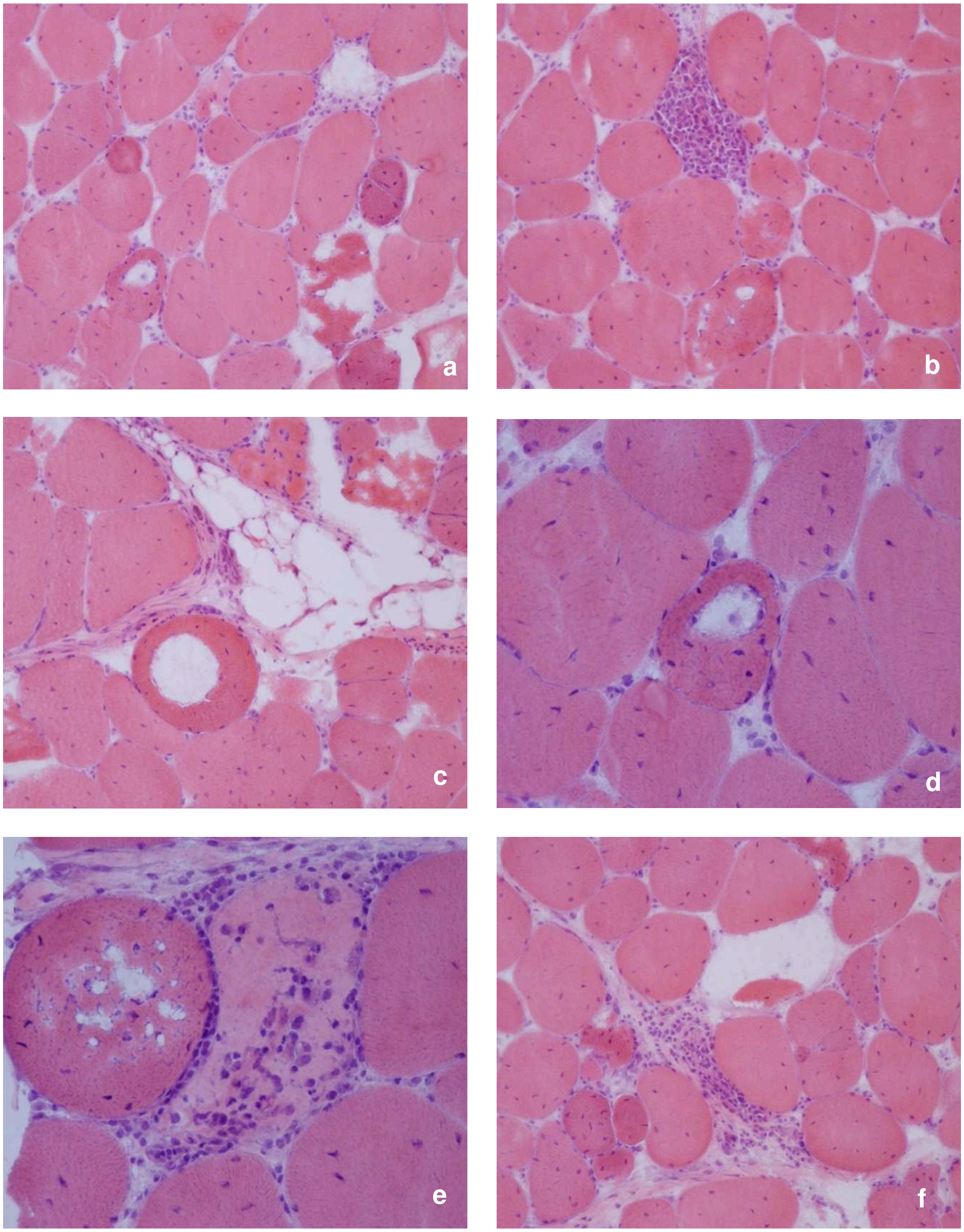


Figure 39. Histo-patolagical analysis of breast muscles. *Figure 39a*: high variability in fibres size, degenerated fibres with vacuoles and necrotic fibres. *Figure 39b*: necrotic fibres with sarcoclastosis. *Figures 39c* and *39d*: degenerated fibres with vacuoles and presence of inflammatory cells. *Figure 39e*: necrotic fibre with inflammatory cells and macrophages. *Figure 39f*: degenerated fibres, presence of inflammatory cells and endomysial oedema.

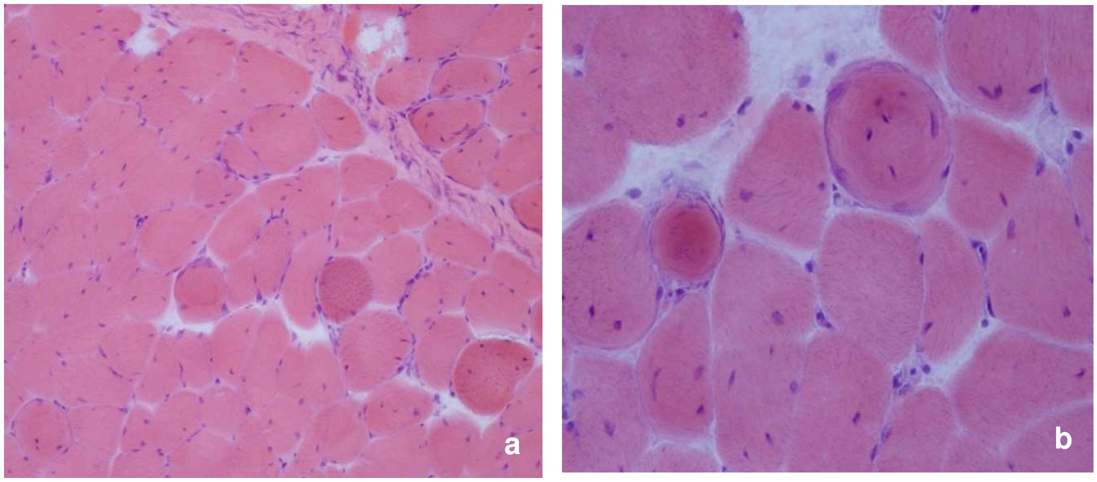


Figure 40. Histo-patolagical analysis of drumstick muscles. *Figure 40a*: high variability in fibres size, necrotic fibres and inflammatory cells. *Figure 40b:* Ringer fibres: myofibrils disposed in a ring. This is suggestive of disorganized myofibrils.

considered in the present study. Genetically determined changes in muscle characteristics could also have had an influence on the susceptibility of these birds to pre-slaughter stressors. As a consequence of the above considerations, additional tests on the muscles affected by this degeneration are required and will be the object of future developments.

In Table 8, information about the incidence of the myopathy are reported. The average overall incidence of the degeneration was 0.47%, with a minimum of 0 and a maximum of 2%. This percentages included both the lesions on breast and leg muscles.

Table 8. Overall incidence of the myopathy and percentages of broilers *Ross 708* and *Ross 308* affected.

	Incidence of IM on *Ross 708* (%)	**Incidence of IM on *Ross 308* (%)**	**Overall Incidence (%)**
Mean	0.4	0.55	**0.47**
SE	0.085	0.13	**0.073**
Min - Max	0 – 1.17	0 - 2	**0 - 2**

Broilers belonging to the genetic line *Ross 308* exhibited a higher incidence compared to the broilers *Ross 708* (0.55% vs 0.4%), even if the two percentages did not differ significantly. In particular, 75% of the flocks composed by broilers *Ross 708* presented an incidence ranging from 0 to 0.6%, whereas 75% of the flocks with broilers *Ross 308* exhibited an incidence ranging from 0 to 0.8%.

8.6 Meat Quality Abnormalities: Dark Firm and Dry (DFD) meat

Another object of the present thesis was the analysis of the incidence of a DFD-like condition on the muscles of the slaughtered broiler chickens.

As described in literature (Lawrie, 1998), a DFD condition can develop in meat as a result of a long-term stress and is characterized by a high ultimate pH (pH>6.3) in the affected muscles. These meats have a restricted application because are prone to microbial contamination even when initially are relatively low microbially contaminated.

Several studies demonstrated that the breast and leg muscles from emaciated birds or submitted to various pre-slaughter stressors had a darker colour, elevated pH and almost completely depleted glycogen (Boullianne & King, 1998; Fletcher, 1999; Niewiarowicz et al., 1977, 1978; Walker & Fletcher, 1993). Stress is the factor that accelerates the metabolism and the quick exhaustion of muscle glycogen supplies. As a result, glycolysis in post-mortem muscle do not occur or is slowed down because the level of substrate (glycogen) of this process undergoes a substantial exhaustion (Lawrie 1998; Owens & Sams, 2000).

8.6.1 Material and Methods

To investigate on the incidence of a DFD-like condition a total of 37650 broiler chickens was chosen randomly from 32 flocks for a period of 7 months (Sep. 2014 - Mar.2015). The broilers belonged to two commercial breeds (*Ross 708 and Ross 308)*, were reared under intensive conditions and slaughtered from 45 to 52 days of age. The average live body weight of the broilers belonging to the breed *Ross 708* was 3.75 ± 0.28 kg (± standard deviation), whereas the broilers of the breed *Ross 308* weighed on average 3.92 ± 0.32 Kg.

The occurrence of the DFD condition was assessed both on pectoral (P) and quadriceps (Q) muscles and pH values were measured 24 h post-mortem on the affected parts by means of a *HANNA® Instruments (USA) pH-211 Microprocessor* pH meter.

The data on the incidence were analysed by descriptive statistics (mean, standard deviation, minimum and maximum values). To study the influence of the external factors on the occurrence of the disease the software JMP v11.2 (SAS Institute) was used.

8.6.2 Results and discussion

Figure 41 shows the macroscopic appearance of breast and drumstick muscles affected by the degeneration object of the present study. The dark colour of the muscles, the firm texture and the high average pH values measured 24h post-mortem (pH around 6.9) allowed to classify this degeneration as a DFD-like condition.

Figure 41. Degenerated breast *(left)* and drumstick *(right)* muscles

Table 9 and Figure 42 summarize the information about the incidence of the DFD-like condition in the inspected broilers.

The overall incidence of the degeneration was 5.74% with a minimum of 0.62% and a maximum of 14.1%. These results were in line with the findings of Mallia *et al*., (2000) that estimated that less than 10% of slaughtered chicken population in Canada exhibited DFD problems.

Ross 308 exhibited a higher overall incidence of DFD problems compared to *Ross 708* (6.95 % vs 4.81%).

Table 9. Incidence of the DFD condition in all the inspected carcasses and incidence on broilers *Ross 708* and *Ross 308.* Mean, Minimum, Maximum and SEM values are also indicated.

	Incidence of DFD on *Ross 708* (%)	**Incidence of DFD on *Ross 308* (%)**	**Overall Incidence (%)**
Mean	4.81	6.95	**5.74**
SEM	0.79	1.1	**0.67**
Min - Max	0.62 – 11.36	1.7- 14.1	**0.62 -14.1**

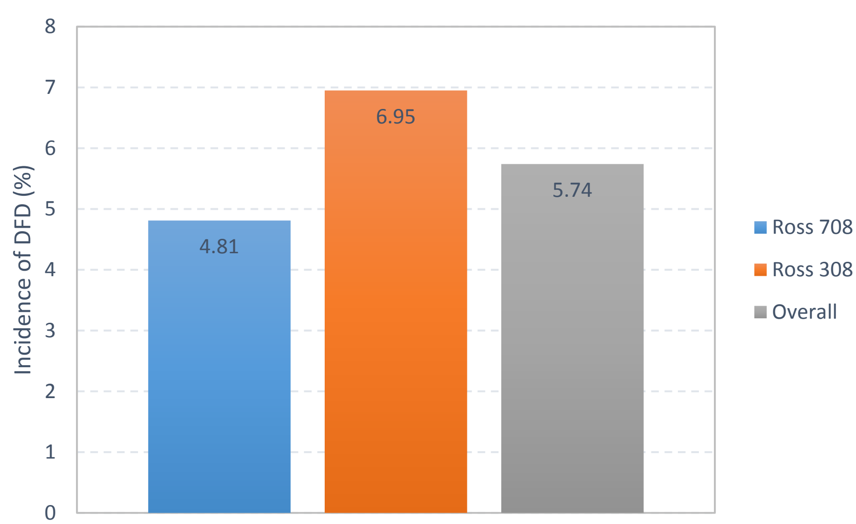


Figure 42. Incidence of the DFD condition in all the inspected carcasses and incidence on broilers *Ross 708* and *Ross 308*.

The statistical analysis did not show any significant effect of the live body weight of the birds on the occurrence of a DFD-like condition ($P = 0.5$), but a significant effect of seasonal factors was found ($P = 0.01$), with a higher incidence of DFD during the cooler month (see Figure 43).

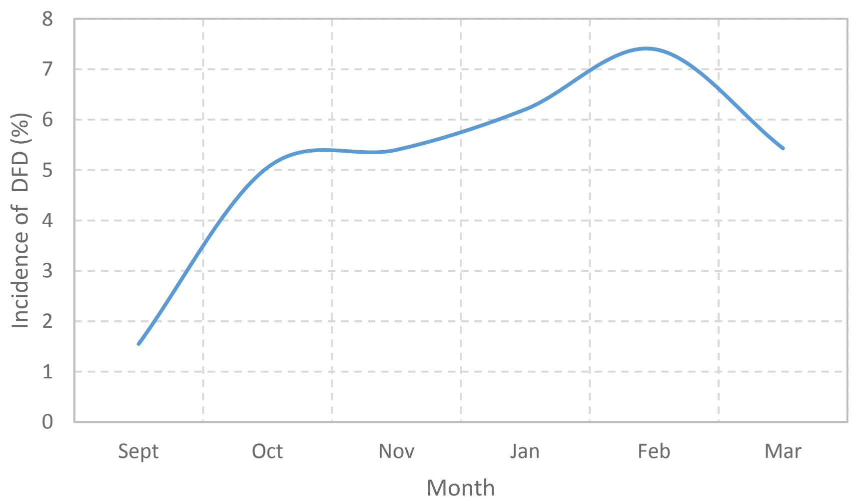


Figure 43. Behaviour of the average monthly incidence of DFD. The incidence was higher during the cooler months.

The statistical analysis also revealed a significant correlation between the occurrence of *Deep Pectoral Myopathy* and the occurrence of a DFD condition ($P = 0.02$). The reason of this interesting finding could be the similar aetiology of these two degenerations, both having a long term stress as a trigger factor.

Summary and Conclusions

The experimental activity of this thesis was carried out at in a major Italian poultry slaughter plant. This represented a peculiar and innovative aspect of the present study, as all the collected data interested a high number of animals and all the results were validated and verified under commercial conditions.

One of the aims of this research activity was the improvement of animal welfare conditions in order to guarantee a "humane" slaughtering to the broiler chickens. In this context, a particular emphasis was placed on the stunning process and on its optimization.

To optimize the stunning operation, which consisted in an electrical multi bird water-bath stunning, different combinations of electrical parameters were tested. In particular, a sine-wave Alternating Current (AC) at frequencies of 200, 400, 600, 800, 1000 and 1200 Hz was used and the voltage was adjusted on the stunner to obtain an estimated average root mean square (RMS) current for each broiler of 100, 150, 200 and 250 mA. The presence of corneal reflex, the occurrence of spontaneous eye blinking and a positive response to comb pinching were used as indicators of the state of consciousness and sensibility of the birds, as result of an ineffective or poor stun.

The statistical analysis showed that the electrical stunning frequency (Hz) and the stunning current level (mA) had a significant effect on the occurrence of animals with suppressed corneal reflex. In particular, as also revealed by a statistical model, an increase in the electrical stunning frequency at a chosen stunning current level produced a decreasing number of birds with suppressed corneal reflex. So, as a consequence, when the stunning frequency was increased, a higher amount of current was necessary to achieve an adequate number of birds without corneal reflex.

The results of both statistical analysis and experimental tests also suggested that, with a given current level, only a narrow range of electrical frequencies could be employed to achieve an effective stunning. This frequency ranges, for each current level, were indicated in this study.

The maximum probability to have unconscious birds was obtained using a current level of 200 mA at 400 Hz or 250 mA at 400, 600 and 800 Hz. These combinations of stunning electrical parameters were also those that produced the lower number of birds with a positive response to comb pinching.

Another aim of the present thesis was the study of the influence of the electrical stunning process on both carcass and meat quality. In particular, the effect of different stunning treatments (with different combinations of electrical stunning parameters) on the occurrence of haemorrhages on carcasses and meat was investigated. Moreover, a macroscopic characterization of these lesions was provided.

The experimental tests showed that the stunning process produced in both pectoral and leg muscles haemorrhages that mainly consisted in *petechiae*, *striae*, *suggillations* or *ecchymosis*. The occurrence of these lesions decreased as the stunning frequency was increased with a reduction of up to 100% for very high frequency levels.

Globally, the lesions revealed more frequent in leg muscles (65%) than in breast muscles (35%). In particular, for low stunning frequency values (up to 600 Hz) and for low current levels (up to 150 mA), the haemorrhages were almost equally distributed in leg and breast muscles, whereas for higher current levels and higher frequency values the haemorrhages in leg muscles became prevalent.

The results also showed that combinations of stunning electrical parameters that produced the highest number of unconscious birds rarely allowed to have carcasses free of haemorrhagic lesions. This finding confirmed a clear limitation of electrical water bath stunning in ensuring both animal welfare and meat quality. This aspect is widely described in literature and several studies have been carried out to overcome this problem. The present thesis aims to represent a contribution in this sense.

The poultry slaughter plant was also used as an epidemiological laboratory to investigate on the incidence and nature of myopathies, degenerations and other abnormalities that frequently affect the carcasses and the meat of broiler chickens.

In this context, the incidence of Deep Pectoral Myopathy (DPM or *Oregon* disease), the incidence of DFD conditions and the nature and incidence of other generic muscular degenerations were investigated.

Experimental tests on 37650 broiler carcasses for a period of 7 months revealed that total incidence of DPM was 4.17%, with a minimum incidence value of 0.94% and a maximum of 8.84%. Tests also indicated that the lesions at an early developing stage were more frequent than those at a late stage, with an average incidence of 2.76% and 1.41%, respectively.

An important role was played by the genetics in the determination of DPM. In fact, broilers belonging to the genetic line *Ross 308* exhibited a higher overall incidence compared to the broilers belonging to the genetic line *Ross 708* (4.75 % vs 3.64%).

Seasonal factors also revealed to have significant effect on the occurrence of the myopathy, with a higher incidence during the cooler months.

A frequent finding at the cut lab of the slaughter plant was also the occurrence of a myopathy, whose features could not be easily attributed to other muscle degenerations described in literature. The histological characterization of this myopathy revealed the presence of increased variability in fibre size, presence of degenerated fibres with invasion of macrophages and fibres with vacuoles. Angular atrophy and necrosis associated with sarcoclastosis were the most prominent finding in pectoral muscles.

Information about the incidence of this myopathy were also reported. The average overall incidence of the degeneration was 0.47%, with a minimum of 0 and a maximum of 2%.

Broilers belonging to the genetic line *Ross 308* exhibited a higher incidence compared to the broilers *Ross 708* (0.55% vs 0.4%), even if the two percentages did not differ significantly.

The occurrence of meat with a DFD-like condition was also a frequent finding. The affected muscles appeared dark in color, with a firm texture and with high pH values (pH > 6.9) at 24h post-mortem. The overall incidence of this degeneration was 5.74% with a minimum of 0.62% and a maximum of 14.1%. These results are in line with earlier findings.

Ross 308 exhibited a higher overall incidence of DFD problems compared to *Ross 708* (6.95 % vs 4.81%). The statistical analysis did not show any significant effect of the live body weight of the birds on the occurrence of a DFD-like condition, but a significant effect of seasonal factors was found. In particular, a higher incidence was observed during the cooler months.

Moreover, the statistical analysis revealed a significant correlation between the occurrence of Deep Pectoral Myopathy and the occurrence of a DFD condition. The reason of this interesting finding could be the similar aetiology of these two degenerations, both having a long-term stress as a trigger factor.

The high incidence of all the above described degenerations and myopathies, which represent an important economic loss for meat processors, can have an explanation in the continuous research for improved body weight gains that has led to the creation of new genetic lines with a rapid muscle growth. This allowed to obtain broilers chicken that can be slaughtered at the age of 45-50 days. These birds, however, often show myopathies, degenerations and lesions that cause the downgrading of the carcasses. Moreover, these broilers are more susceptible to environmental conditions and stressful situations that can represent trigger factors for the occurrence of this kind of lesions. In this sense, a better protection of animal welfare during the different phases of the food chain (hatchery, livestock, transport and slaughtering) seems essential.

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