CHAPTER 8

Summary and discussion
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This thesis aimed to identify metabolic parameters that may be used as indicators for welfare during pre-slaughter treatments and subsequent meat quality in broiler chickens. Because physical pre-slaughter conditions and stress responses increase the energy demand and meat quality is affected by the energy level in the muscle, and because energy is available only in a limited quantity from the moment feed is withdrawn at the farm, metabolic parameters at various levels were measured. Stunning before slaughter and exsanguination cause a rapid depletion of the readily available energy and prevent resupply of energy rich compounds and oxygen, and this causes epileptic and ischemic conditions in the brain. Because such conditions result in brain damage and ultimately in unconsciousness, which is desirable from a welfare perspective, measurement of metabolic failure was studied. Brain impedance recordings were chosen as a means to measure metabolic failure in the brain, which results in a reduction of the extracellular space through failure of the ion pumps in the cell membrane. Chapters 2, 3, and 4 described the development of this indicator for poultry and worked towards identification of the criteria that indicate an effective stun. Feed deprivation and other pre-slaughter treatments result in a depletion of available energy. The rate of depletion depends on the specific conditions the chicken is submitted to. Liver glycogen is quickly mobilized to maintain blood glucose levels for energy supply. As such, liver glycogen was used to study short term demands of energy. A decrease in blood glucose is then a parameter that may indicate a shift in the energy metabolism. An ultrafiltration-collection device as described in chapter 5 was chosen to monitor metabolites in blood. Early post mortem energy metabolism in the breast muscle depends strongly on the size of its glycogen stores at slaughter and the rate of energy depletion thereafter. In chapters 6 and 7 muscle metabolite levels were chosen as parameters to survey the early post mortem metabolic processes and their usefulness as indicators of meat quality.

Metabolic parameters

Brain impedance recordings

In chapter 2 the development of brain impedance recording as a method for measuring brain metabolic activity in poultry was described. This method was developed as an alternative to measuring brain electrical activity in an attempt to come to a more unambiguous and sensitive determination of the onset and development of unconsciousness after stunning. Measurements found under ischemic conditions after inducing cardiac arrest in broiler chickens were similar those found in other species: an increase in impedance from 1 min. after cardiac arrest that continues up to 186.2% after 10 min. According to Maxwell's equation this corresponds with a decrease of the extracellular volume by 46.3%. As a decrease of the extracellular volume reflects cellular swelling due to failure of the
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Energy requiring systems that should maintain the ionic gradients across the cell membrane, i.e. the Na⁺/K⁺ pump. Thereby the ability of signal transduction disappears, and we may assume a state of brain metabolic failure and unconsciousness. This measurement proved to be reliable regardless of a previous 12 h period of fasting. Previously fasting was reported to reduce the hyperglycemic response and protect against hypoxia ischemia in rats (Dijk et al., 1994). Hypoxia was also shown to cause a decrease in rat striatal extracellular glucose (Kuil and Korf, 1991), which might then demand a hyperglycemic response. The glycogen body, a uniquely avian structure in the lumbosacral sinus of the spinal cord that consists of glycogen rich cells, has a putative metabolic function in neuronal support of the central nervous system. This could prevent the need for a cerebrally regulated hyperglycemic response, but it would also keep open the possibility for glutamate release originating from the metabolic pool during ischemia.

While metabolic failure caused by ischemia after cardiac arrest occurs gradually, an epileptiform insult, as can be generated by electrical stunning, causes an immediate and massive depolarization of neurons and a depletion of local energy sources through increased glycolysis. The application of brain impedance recording under such circumstances was tested and described in chapters 3 and 4. In chapter 4 the non-metabolic parameters heart rate and blood pressure were measured as well as to get additional information about the occurrence of ischemic conditions in the brain. A range of responses was found, depending on stunning method and voltage. Brain impedance remained at base level for much longer following exsanguination by cutting one jugular vein without stunning (4 min) than following the induction of cardiac arrest (1 min). This suggests that as long as cardiac function and the afferent blood vessels remain intact poultry brain function is not metabolically compromised. Brain impedance was found to increase immediately after stunning in some cases, reflecting a decrease of the extracellular space due to immediate brain damage. But this was not uniformly found. Data suggested an effect of stunning voltage and electrode position. The induction of cardiac arrest or fibrillation was suggested to be important, as an immediate and progressive increase in brain impedance was only uniformly found in combination with a rapid stop of the circulation. Brain impedance increased faster if an immediate increase following stunning was found. These aspects confirm the presence of damaged brain tissue, which is most effectively induced if both immediate epileptic and ischemic conditions are present. We concluded that brain impedance recordings were an effective tool for measuring brain metabolic activity after electrical stunning, with an immediate increase indicating the immediate onset of unconsciousness.

Liver glycogen and pH

The liver glycogen store is the first energy store to be addressed to maintain blood glucose homeostasis. This store will be metabolized already after a few hours of feed deprivation in broiler chickens and can be completely exhausted
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after 6 h, although residual glycogen levels have been found after 10 h. This process occurs without affecting glycogen stores in skeletal muscle (Warris et al., 1988; Uijtenboogaart et al., 2001). Consecutive acute stressors increase blood glucose, which after an initial peak is accompanied by a decrease in liver glycogen levels (Donaldson et al., 1991). As a metabolic parameter liver glycogen is useful to measure changes in the short term metabolism. A drawback of this parameter is that it is difficult to measure other than in freshly killed animals, and even then the procedure must be completed quickly because liver glycogen is being actively mobilized at death. As an indirect and easier measurement of residual liver glycogen liver pH can be used (Warris et al., 1988; Wal et al., 1999). A low pH reflects a relatively high residual glycogen at the time of death and vice versa. As such we chose liver pH as a parameter to study the effects of feed deprivation, transport and stunning methods on the chicken’s metabolism in chapters 6 and 7. A period of 5 to 7 h of feed deprivation resulted in a difference in liver pH of 0.2 units on average, indicating the normal metabolization of energy stores, which cannot be replenished. No indication of an increased energy demand due to stunning of transport on liver pH was found. We concluded that liver pH is a good parameter to measure short term changes in energy supply, but that this factor outweighed any potential effects of acute stressors like transport.

Blood glucose and lactate

Glucose and lactate concentrations in blood are quite stable under most circumstances (glucose: 12.7 mM, lactate: 4.9 mM), although several factors affect these concentrations within the margins of homeostasis (Sturkie, 1986). A day-night rhythmicity may exist (Savory, 1987), although in high metabolic chickens like broilers and broiler breeders fed ad libitum this rhythmicity may have disappeared (I. de Jong, 2001, ID-Lelystad BV, P.O.B 65, 8200 AB Lelystad, The Netherlands, pers. comm.). Under the influence of catecholamines and glucagon glucose levels may increase after acute stress (Armario et al., 1990; Donaldson et al., 1991). Feed deprivation leads to a slight decrease in blood glucose after 2 h, which drops to a 10 to 15% decrease overnight. Refeeding can restore blood glucose to normal values within half an hour after consuming even small amounts of feed (Savory, 1987). After depletion of the liver glycogen store, blood glucose levels will be maintained by gluconeogenesis and glucose sparing. A shift to fat metabolism will occur, which has its peak between 24 and 72 h of fasting (Sturkie, 1986). Feed availability dependent glucose responses are interrelated with the somatotropic and thyrotrophic axis. Decreased levels of T₃ and increased levels of growth hormone following feed deprivation confirm a time dependent coregulatory effect of the lipid metabolism (Buyse et al., 2000), which should be taken into account in interpreting glucose measurements. In this thesis we chose to measure blood glucose and lactate concentrations to study the effects metabolic exhaustion and the metabolic response to acute stressors.
Changes in time of glucose level or rhythmicity may be studied with a good monitoring tool. Chapters 5 and 6 describe the development and application of a novel ultrafiltration-collection device (UCD) that is able to remotely and continuously sample blood ultrafiltrate. The ultrafiltrate could later be analyzed offline on glucose and lactate content. Using this method we were able to generate 8 h glucose and lactate profiles with a resolution of one measurement per 5 minutes of sampling time. Unfortunately the current design still contains a few flaws. While the 8 h profiles demonstrate that the method works on principle, the glucose and lactate concentrations that were measured increased to supraphysiological levels with longer sampling times both in vitro and in vivo. Apparently water diffused through the wall of the collection tubing. As the rate of diffusion was unknown, the proper values could not be reconstructed and presupposed effects of feed deprivation could not be discriminated. However, taking only the most recent 30 min sample that has minimal diffusion high correlations with blood samples taken during exsanguination were found. In the profile no changes corresponding to the periods of transport were found. Recommendations for improvements in construction and materials have been made to prevent diffusion of water through the wall of the collection tubing, and to reduce longitudinal diffusion of the sample. With these improvements the UCD may become a widely applicable tool for the stress free monitoring of glucose and lactate in chickens and other species, in blood and other body compartments.

In addition blood samples were taken during exsanguination and analyzed on glucose and lactate concentrations, which were 12.7 mM and 4.9 mM on average respectively. Decrease in blood glucose of 1.2 mM on average was found after a 5 h period of feed deprivation. This decrease was more pronounced in 5 weeks old than in 6 weeks old broiler chickens. This might be explained by a larger glycogen storage capacity in heavier chickens. Overall blood glucose remained much higher than the hypoglycemic levels at which pathological symptoms and clinical stupor occur (2-3 mM) accompanied by δ-waves on the EEG. Blood glucose levels below 1 mM were reported to cause an isoelectric EEG (Auer and Siesjö, 1993). Until slaughter glucose supply to the brain was not compromised in the experiments described in chapters 6 and 7. Acute stressors and exercise are known to increase glucose mobilization through catecholamine and glucagon secretion and neural feedback, respectively (Brackenbury and Vincent, 1988; Donaldson et al., 1991; Kjaer, 1998). Following electrical stunning blood glucose was found to be 1.0 mM higher when head only stunning was applied than after the application of whole body stunning. This may be explained by high muscle activity during the clonic convulsions that occur with head only stunning creating an increased demand for glucose. The short burst of activity may not last long enough to consume the extra glucose released by the liver, resulting in increased blood glucose levels. Blood samples were taken too quickly after stunning for muscle lactate production to show. In chapter 6 no effects on blood glucose or lactate of 1.5 h transport under relatively good conditions were found. Next to the absence of
stressful climatic conditions, it is unclear whether this kind of transport causes only mild transient stress or whether the response to transport is negligible compared to that of other stimuli. Contradictory results on glucose response to transport have been reported before (Halliday et al., 1977; Freeman et al., 1984; Warris et al., 1993), confirming a need for the exact specification of the conditions in transport studies, and a knowledge gap in the actual impact of the respective potentially stressful stimuli that occur during transport. We conclude that blood lactate is a good indicator of increased energy demand during fasting and that it can be a good indicator for acute stress (Savory, 1987; Armario et al., 1990; Donaldson et al., 1991) and a sudden increase in muscle activity, which might also increase blood lactate in living animals. A response to chronic stress is much more difficult to interpret because of possible changes in demand, interaction with the lipid metabolism and a feedback effect of glycemic status to hypothalamus-pituitary-adrenal axis activation (Goeij et al., 1992; Buyse et al., 2000; Puvadolpirod and Thaxton, 2000).

Muscle metabolites

After death energy reserves in the muscle rapidly degrade and result in the onset of rigor mortis once less then 20-30% of the normal ATP can be generated (DeFremery, 1966). The state of contraction at rigor affects the toughness of the meat. The relationship between the onset of rigor mortis and meat quality has been studied (Papinaho and Fletcher, 1996; Kang and Sams, 1999), but the precise course of the early postmortem metabolism in the breast muscle needed to be further clarified to indicate how processing can be optimized. The major metabolites in the glucose metabolic pathways in the breast muscle studied were glycogen, glucose, ATP and lactate concentrations as well as the pH and R-value, which is the ratio of inosine to adenosine compounds. In chapters 6 and 7 these parameters were studied at several moments early postmortem to explain the mechanism of metabolic degradation.

Muscle glycogen is the main store of energy in the muscle after death. Glycogen levels remain high regardless of pre-slaughter feed deprivation, but they are influenced by recent muscle activity (Grey et al., 1974; Edwards et al., 1999; Uijttengoogaart et al., 2001). Data in chapters 6 and 7 confirmed these findings, as no effect of feed deprivation was found, but stunning methods that caused clonic convulsions did reduce muscle glycogen content. In unstimulated muscle glycolytic activity ceases after 6 h postmortem. Intermediate metabolism may continue a little while longer as indicated by a further increase in R-value between 6 and 48 h (Schreurs, 1999). Up to 2 h postmortem muscle metabolism is fairly stable, although produced lactate will not be removed and its accumulation may cause a slight decrease in pH. After 2 h glycolytic metabolism degrades rapidly. After 4 h ATP generation through glycolysis stops, probably due to a decrease in pH below about 6.2. Residual ATP will be metabolized. Glycogenolysis will metabolize any glycogen that is left, which results in residual glucose levels at 6 to 8 h
postmortem. Normal processing results in an ATP decrease of about 50% after 1 h and a rigor induction at 4 h postmortem. Only stunning methods causing severe clonic convulsions rigor was inducers already after 2 h. For a faster induction of rigor additional treatments, e.g. electrostimulation, must be applied (Craig et al., 1999; Sams, 1999), although the effects of a rapid decrease affecting the metabolic processes and muscle pH must be taken into account. We conclude that measuring muscle metabolites provides further insight in the early postmortem processes and are useful for studying these processes in relation to meat quality. Also, except in the case of recent, intense muscle activity, substrate availability is not a limiting factor in the postmortem metabolism in the glycolytic breast muscle. Instead pH is the factor that determines when glycolysis stops and the energy supply is limited to the intermediate metabolism. Too rapid pH may result early protein denaturation, causing PSE-like (pale, soft, exudative) conditions in the meat (Barbut, 1997; Wilkins et al., 2000).

Indicators for animal welfare and meat quality

Brain impedance recordings and stunning

An effective electrical stun induces immediate unconsciousness that lasts until the death of the animal by exsanguination. Unconsciousness is a result of brain damage induced by a generalized epileptiform insult and ischemia. Loss of brain function is usually measured by electroencephalography. In poultry epileptiform brain activity differs from a generalized epileptiform insult as found with humans and mammalian animals, making the analogous assumption of unconsciousness invalid. Either the disappearance of somatosensory evoked potentials (SEPs) or the occurrence of an isoelectrical line is used as the criteria when the damage to the brain is such that we may safely assume unconsciousness. However, these criteria rarely occur immediately after stunning, and it was therefore suggested that to ensure unconsciousness it would be necessary to have a circulatory stop by inducing cardiac arrest or fibrillation as well (Gregory and Wotton, 1987).

We demonstrated in chapters 2 and 3 that brain damage could also be measured by brain impedance recordings. Both with head only stunning and whole body stunning we found chickens with an immediate and progressive increase in brain impedance, which reflects immediate and lasting brain damage. This damage is such that a large shift in ion gradients prevents signal transduction (Harreveld, 1966). We may then conclude that the chicken is unconscious and the stun was effective. We therefore have an indicator that is a useful alternative to the electroencephalographic indicators used so far. However, like the electroencephalographic indicators brain impedance recordings are conservative measurements. While an increase in impedance may be associated with
unconsciousness, it can not be said that in absence of an increase in impedance the animal is conscious. The consequence for evaluating electrical stunning methods is that only conditions can be identified under which a stun is certain to be effective, but not the minimum conditions that consistently result in the onset of unconsciousness in all chickens.

For stunning and killing methods other than electrical brain impedance may be a less effective indicator or not useful at all. While the effectiveness of gas stunning depends on the composition of the gas mixture used and the method of application, the effects are primarily caused by hypoxia followed by ischemia once the heart stops. The shortest period in which a significant increase in brain impedance was found under purely ischemic conditions after cardiac arrest in chapters 1 and 3 was 1 min. After exsanguination without prior stunning it lasted 4 min. until brain impedance increased. With gas stunning any time period in between these figures may be expected. Loss of SEPs occurred on average at 24 and 47 s using an 30% Ar/60% CO₂ in air, and a 40% CO₂/30% O₂/30% N₂ gas mixture, respectively (Raj et al., 1998). Measuring SEPs then appears to be a more sensitive measurement to evaluate the loss of consciousness for gas stunning methods than brain impedance. Brain impedance recordings can not be used with captive needle stunning. The shot damages the tissue and may change the relative position of the impedance recording electrodes. Also the total brain volume can not be assumed to be constant and the resulting changes in brain impedance may deviate from their proper values as Maxwell’s equation can no longer be applied.

Liver pH and blood metabolites

Liver pH and blood glucose concentrations change within a short time after feed withdrawal. Feed deprivation will ultimately lead to energetic exhaustion and the chickens will develop problems maintaining their homeostasis. In animals with such a high metabolism as broiler chickens, liver glycogen can be completely exhausted after 6 h of feed deprivation (Warriss et al., 1988). Before that time blood glucose levels start to decrease (Savory, 1987), suggesting a reduced rate of glucose mobilization by the quickly available hepatic glycogen source. However, the lack of a further reduction of blood glucose levels indicates that broiler chickens are able to mobilize glucose from other sources. Gluconeogenesis is an important alternative source of glucose, in the context of this study particularly so if it uses muscular protein as a substrate resulting in lower yields (Veerkamp, 1986).

In chapters 6 and 7 we found a 15% glucose decrease in blood samples taken during exsanguination in chickens that had experienced a 5 h period of feed deprivation as well as differences in liver pH. A period of 1.5 h of transport during which feed was not available did not have any effects. Blood glucose and liver pH are good indicators of changes in the hepatic route of glucose metabolism. However, it is difficult to associate a reduction of the hepatic pathway solely as an indicator for welfare. Blood glucose levels after 5 to 24 h of feed deprivation are still high enough to maintain a basal resting metabolism. A severe hypoglycemia is an
indicator for compromised welfare and pathological risk (Auer and Siesjo, 1993). During a 24 h period of fasting the demand for food becomes more inelastic (Faure and Lagadic, 1994), and feed deprivation has been reported to elicit a stress response (Nicol and Scott, 1990). With high metabolic animals like broiler chickens this could occur within short periods of feed deprivation (Knowles et al., 1995).

Additionally, an increase in blood glucose is a normal reaction to an acute stressor, mostly as a result of an increase in catecholamine and glucagon levels, to prepare the body for performing coping behavior (Donaldson et al., 1991; Beuving and Blokhuis, 1997). As such, blood glucose is an indicator for acute stress. However, care must be taken with interpreting changes in glucose levels, as they are influenced by other metabolic pathways as well (Puvadolpirod and Thaxton, 2000). Additionally, if blood glucose levels are reduced and could not be increased as a response to an acute stressor, this is an indicator of chronically compromised animal welfare. In this study we did not find any glucose response to 1.5 h of transport, but we did demonstrate an effect of stunning method. Head only electrical stunning resulted in higher blood glucose than waterbath stunning. Whether this effect was caused by an increased demand due to the clonic muscle activity or suffering from a bad stun or both, it demonstrates that the chickens were capable of responding with an increase mobilization of glucose to the blood. In this experimental set-up it can be concluded that blood glucose can be used to study acute effects, but does not imply a condition of complete metabolic exhaustion.

A decrease in blood glucose levels does not necessarily imply a reduction of energy available to the brain. Not only can the brain use other substrates, e.g. ketone bodies, for energy, but also the glycogen body which lies in the lumbosacral sinus of the spinal cord is thought to play a role in providing the brain with glucose through the cerebrospinal fluid (Sturkie, 1986). The high glycolytic activity in this organ suggests that it is not only activated if other sources fail to provide sufficient glucose, but is always active. Its behavior during short and prolonged periods of feed deprivation is unknown.

**Muscle metabolites**

The early postmortem muscle metabolism determines the moment of onset of rigor mortis (Schreurs, 1999). This has implications for carcass processing. Deboning before a state of rigor has been reached will result in an increased toughness of the meat. Also a very rapid exhaustion of muscle energy due to a high muscle metabolism causes a similarly rapid decrease in pH, resulting in changes in meat color and water holding capacity (Barbut, 1997; Wilkins et al., 2000). The state and rate of early postmortem metabolism was studied by measuring the metabolic parameters glycogen, glucose, ATP, lactate, pH and R-value over time. The interrelated changes of these parameters were evaluated as indicators for breast meat quality.
Muscle glycogen was found to only affect meat quality if it is a limiting factor. A fast consumption of muscle glycogen, e.g. by recent excessive muscle activity, results in a rapid accumulation of lactate and a decrease in pH with the aforementioned effects on meat color and water holding capacity. If glycogen is not limiting, ATP generation through anaerobic glycolysis will come to a halt while glycogenolysis continues after this moment until all glycogen has been converted into glucose. This results in residual glucose levels. Muscle glycogen and muscle glucose concentrations are therefore not very useful as indicators for meat quality. Glycogen will never be present in the muscle after 6 h postmortem or sooner, depending on previous treatments. Similarly, there will most likely be residual glucose in the muscle, the amount of which depends on the residual glycogen at the moment glycolysis stops, which is not easily predictable. Muscle lactate and muscle pH correlate quite well, which is the result of the simultaneous production of lactate and H+ by anaerobic glycolysis. These parameters are also useful as indicators for meat quality only in extreme situations when the pH decreases so fast that it can denature proteins and results in changes in meat color and water holding capacity. For use in practice muscle pH is the preferred parameter of the two, because it is much easier to measure than lactate concentration. ATP levels have long been recognized as an important indicator for the onset of rigor mortis. A threshold value of 20% was given as the moment when rigor sets in (DeFremer, 1966). The R-value, which is the ratio of inosine to adenosine compounds, closely but not exactly follows the ATP concentrations. In particular in the tail of the post mortem metabolic degradation the R-value continues to increase when ATP can no longer be generated, because lower energy adenosine compounds (ADP, AMP) may still exist. The R-value is an easy to measure parameter that is an indicator of the residual metabolizable energy in the muscle and may be used as an indicator to predict effects on meat toughness in relation to deboning time.

Muscle metabolites are not very suitable as indicators for animal welfare in the situations described in this thesis. Whereas feed deprivation and acute stressors induces early changes in blood metabolites, they do not affect muscle metabolites (Warriss, 1988; Uijttenboogaart et al., 2001). Muscle lactate might give additional information when it concerns responses to exercise related stress situations, like postural compensation as a response to vibrations during transport (Scott, 1994).

**Metabolic parameters as indicators for animal welfare and meat quality**

The causes for a reduction in animal welfare or meat quality have been shown to have a metabolic component. This component includes the possibility to maintain the metabolic needs under normal or extraordinary conditions and for exercise, metabolic consequences of hormonal and behavioral stress responses, and metabolic responses to traumatic events. In this thesis we studied several parameters to see whether they could be used as indicators for changes in this
metabolic component and (potential) subsequent reductions in animal welfare or meat quality.

It was demonstrated that metabolic parameters show metabolic changes to 5-7 h feed deprivation and stunning method, but not 1.5 h transport. Conclusions that animal welfare was reduced under the conditions described in this thesis could not be drawn from these parameters. However, the data suggests that there will be risks under less mild conditions than applied here. Meat quality was reduced as a result of metabolic changes early postmortem only. Early postmortem metabolism or treatment did not affect ultimate meat quality parameters, provided deboning was done after the onset of rigor mortis.

Implications for the treatment of poultry before slaughter

Feed deprivation

To prevent fecal contamination of the birds and the crates a period of feed deprivation of 5 h before transport is recommended. In this thesis changes liver pH and blood glucose were measured to study the effects of this treatment. Differences in liver pH and blood glucose were found between 5 h feed deprived chickens and chickens fed until transport. Blood glucose was such that chickens were not in a state of severe hypoglycemia. Combined with the differences in blood glucose levels found after different electrical stunning methods, we found that chickens were able to generate a glucose response to a stimulus, in this case increased muscle activity. No effects of feed deprivation on meat quality were found. We therefore conclude that, while feed deprivation for 5 h affects the metabolism, these effects can not be said to be a compromise to animal welfare or meat quality based on the measured parameters. A reduced welfare due to frustration of feeding behavior or a hunger feeling can not be excluded, but was not studied here.

In practice, however, much longer periods of feed deprivation were found. Ultimately this will result in energetic exhaustion. The inability to maintain homeostasis or perform normal coping response compromises animal welfare. In high metabolic animals with a need for regular feed consumption, feed deprivation can become a stressor. Also, high stress levels are known to affect meat quality and result in PSE meat. The criteria that define the moment when feed deprivation lasts long enough to become a welfare and subsequently a meat quality problem are not well known and further research is recommended.

Catching and crating

While catching and crating have not been the subject of this study, these treatments are known to be stressors that can also affect meat quality. These treatments were necessarily part of the pre-slaughter treatments in chapters 6 and
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7. The acts of catching and crating are acute stressors, but the consequences of being crated have an impact until removal from the crate and shackling. Metabolic parameters may change as a result of the stress response and could be measured to study the impact of these treatments. If bruises and fractures sustained during catching or crating, they are felt until slaughter, and social and climatic stress remain present until removal from the crate. Novel environment and movement are inherent to the current system and can not be avoided as long as transport from farm to slaughter plant is necessary.

Transport and lairage
Transport is a very complex process. Stimuli that chickens will experience during transport and lairage include novel physical and social environment, climatic changes, crowding, vibration, acceleration, noise, and prolonged deprivation of feed and water, with the duration depending on the distance between the farm and the slaughter plant and traffic conditions. How much welfare is reduced depends on the exact circumstances. In practice, however, a certain percentage of dead-on-arrivals is not uncommon (Bayliss and Hinton, 1990). No effects of transport on chicken metabolism, muscle metabolism or meat quality were found in this thesis. But transport conditions described in chapter 6 were more lenient than those in commercial practice, with a lesser density of chickens per crate and crates per truck, good climatic conditions, and a transport duration of only 1.5 h. We can therefore not conclude on the basis of these data that transport under these conditions was a severe stressor to affect meat quality. Given the complexity of transport we can not conclude that the chickens did not experience any stress either. To ensure the absence of transport stress altogether would require the absence of transport of live animals. While on-farm slaughter is not a new idea (Kettlewell and Turner, 1985) and has its merits regarding animal welfare, problems regarding logistics, meat quality and production costs are to be expected and need further research and development in order to make this a viable option in commercial practice.

Shackling, stunning and killing
The large slaughter plants slaughter 9000 animals per hours these days. In order to achieve such rates, the process is highly automated. Uncrating and shackling is one of the few processes still done manually. This procedure is unavoidable with the current method of transport, stunning and killing. It is reported to be a stressful procedure (Kannan et al., 1997) and therefore an unavoidable stressor within the restraints of the current slaughter process. The different stunning and killing methods described in chapter 7 have different requirements. With captive needle stunning shackling may be avoided, but some form of fixation and positioning of the head for the needle to give a good stun is needed. Fixation in a cone has been studied with good results (Hillebrand et al., 1994). Both gas stunning methods require no form of shackling or fixation at all. The container can
automatically be emptied onto a conveyor belt, which enters the stunning apparatus, or the chickens can be stunned while still in their container (Raj, 1997).

Whether the conventional waterbath stunner can provide an effective stun and which settings are optimal has long been cause for debate. From a welfare point of view we defined a good electrical stun as one that immediately induces unconsciousness, which lasts until the death of the animal by exsanguination. From a production point of view a good stun immobilizes the chicken for easy killing, optimal bleed out, and does not affect meat quality negatively. The optimal settings of the stunner for either point of view are in conflict. Low currents avoid muscle supercontraction and thereby prevent hemorrhages and broken bones. High currents have been reported to benefit the induction of lasting unconsciousness. With measurements of brain electrical activity it is difficult to determine whether the onset of unconsciousness is immediate and we have to rely on conservative parameters like the disappearance of SEPs or the occurrence of an isoelectrical line. With brain impedance recordings immediate unconscious was assumed when the impedance was increased immediately after stunning and continued to increase. While this occurred more often with high currents, as described in chapter 4, current did not determine stunning effectiveness alone. With some chickens that were head only stunned with currents up to 400 mA we did not find an immediate increase in brain impedance. A consistent immediate increase was found only with 150 V (corresponding with an average of 166 mA) whole body stunning, At which setting all chickens also experienced cardiac arrest or fibrillation. Although it can not be concluded that at other settings stunning efficiency is less, this is the minimum setting at which we can ensure an effective stun for all animals. As a stunning method the conventional waterbath can therefore be effective, provided that the stunning current is increased to 150 mA per bird, which is higher than the European recommendations and the Dutch legal requirements.

With gas and captive needle stunning brain impedance recordings can not be applied as easily, so different measurements are required to determine their effectiveness and humaneness. For captive needle stunning the same criteria for the induction of unconsciousness can be applied and these can be met (Hillebrand et al., 1996). With gas stunning the onset of unconsciousness can never be immediate and other criteria need to be established with regard to the time until the onset of unconsciousness and possible suffering in the period before.

Stunning methods are well known to affect carcass and meat quality. This was also found in chapter 7 of this thesis. Electrical stunning methods resulted in higher hemorrhage scores and lower L* values than captive needle and gas stunning methods. Comparing with whole body electrical stunning head only stunning resulted in lower shear force when the chicken was allowed to freely flap its wings during the application of the stun. Fixation in a funnel negated this effect. In addition head only stunning resulted in lower water holding capacity up to 8 h postmortem, but this effect was negated after 24 h. Head only stunning also

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significantly had a faster postmortem glycolytic rate. On ultimate values, however, postmortem energy metabolism had no effect on meat quality if deboned after rigor had set in. After 24 h no differences between stunning method were found, except for color. Other studies have found differences between different stunning methods, but it is more likely that these must be attributed to processing factors rather than the postmortem muscle metabolism.

Concluding remarks

This thesis aimed to identify metabolic parameters that may be used as indicators for welfare during pre-slaughter treatments and subsequent meat quality in broiler chickens. Additionally pre-slaughter treatments were evaluated using these indicators. We found that brain impedance recordings can be used as a parameter for measuring brain metabolic failure. Brain impedance recordings were demonstrated to be a sensitive tool for determining unconsciousness immediately after electrical stunning. As such it is a good indicator for the humaneness of, especially electrical, stunning methods. Based on the data presented in this thesis, a setting of 150 V (150 mA per chicken) is a minimum level if the immediate induction of unconsciousness is to be guaranteed. It is observed that this recommendation exceeds the minimum settings required by Dutch and recommended by European law.

Blood glucose and liver pH are useful parameters to determine a period of feed deprivation of at least 5 h. Given the maintenance of a minimum blood glucose level this parameter is not a good indicator for chronic stress, except if glucose drops below this level in the case of complete energetic exhaustion. However, the experiments described in this thesis such conditions did not occur. Blood glucose may be a good indicator for acute stress, and blood lactate may be an indicator for activity-related stress. Short transport under mild conditions could not be said to be an acute stressor based on the data described in this thesis.

Muscle metabolism is fairly independent of the metabolism in other parts of the animal. The metabolic rate in the early postmortem muscle strongly depends on recent muscle activity. Stunning method is a major cause for muscle activity. Clonic convulsions will increase the metabolic rate, while tonic convulsions or muscle relaxation will not affect muscle metabolism much. No effects of restricted feed deprivation and short transport on muscle metabolism were found. Muscle metabolism has a negative effect on meat quality only if the carcass was deboned prior to the onset of rigor mortis, which must be considered for further processing.
REFERENCES


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